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Static magnetic field pretreatment affects early steps of chlorophyll biosynthesis in soybean seedlings under arsenic and mercury toxicity

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ABSTRACT

The current work examined the effects of static magnetic field (SMF) on biomass and chlorophyll metabolism in seedlings exposed to arsenic (As) and mercury (Hg) toxicity. Soybean (Glycine max) seeds were magnetoprimered using static magnetic field of 200 mT for 1h. The detrimental effects of As and Hg toxicity on seedling growth were demonstrated by the decrease in biomass of unprimed seedling grown in different concentrations of sodium arsenate (0.0, 0.025, 0.075, and 0.1 mM Na arsenate) and mercuric chloride (0.0, 0.05 and 0.1mM HgCl₂). Both the stresses were found to decrease the chlorophyll content and δ -amino levulinic acid synthesizing activity (ALAS) as well as δ -aminolevulinic acid dehydratase (ALAD) activities. However, magnetopriming has been shown to be successful in reducing the adverse effect of these stresses. The total chlorophyll content was increased by magnetopriming seem to be due to enhancement in enzyme activities involved in early steps of chlorophyll biosynthesis such as ALA synthesizing and ALAD activities in soybean seedlings under both the stresses. The positive effects of magnetopriming enhanced the tolerance index (in terms of seedling dry weight) towards arsenic and mercury toxicity during early seedling growth of soybean may be due to the enhancement in the levels of carotenoids and chlorophyll, as well as the activation of the enzymes involved in chlorophyll biosynthesis. These findings suggested that toxicity caused by Na arsenate is more toxic than HgCl₂ and magnetopriming can lessen the adverse effects of As and Hg toxicity in soybean seedlings and increase the tolerance to these heavy metals.

Keywords: Arsenic, chlorophyll, mercury, static magnetic field, toxicity.

Plants are often stressed by abrupt changes in their environment and encounter number of stresses which affect the crop productivity worldwide. Contamination of soil and water with pollutants is accelerating at an

alarming speed all over the globe (Mishra et al., 2023). One of the major contaminants are supposed to be heavy metals (HM) which cause considerable decline of crop growth and productivity (Prajapati et al., 2020).

Moreover, several health issues are reported due to these heavy metals as they easily find their way to human body through food chain (Zaynab et al., 2022). Among all the heavy metals, As and Hg are recognized as the top hazardous metals/metalloids (Ali and Gill, 2022). Negative effects of arsenic and mercury on plants include inconsistent germination, reduced biomass accumulation, inhibition of nutrient and mineral absorption, loss of photosynthetic pigments, decreased chlorophyll biosynthesis, impaired photosynthesis and disturbances in water uptake and protein function (Jain and Gadre, 2004 a,b; Gworek et al., 2020).

The synthesis and concentration of chlorophyll pigment plays a central role in plant photosynthesis, directly influencing the photosynthetic rate of leaves. In plants, chlorophyll biosynthesis is affected by diverse environmental factors, such as, heavy metal toxicity, salt and drought stress (Jain and Gadre, 2004 a,b; Turan and Tripathy, 2015; Tewari et al., 2021). The biosynthesis of chlorophyll involves a complex pathway (Tanaka and Tanaka, 2007; Gupta et al., 2013), where the major steps are conversion of glutamate to δ -aminolevulinic acid (ALA), formation of porphobilinogen from ALA, insertion of magnesium into proto IX for formation of Mg-proto IX, transformation of protochlorophyllide into chlorophyllide *a* and esterification of Chlorophyllide *a* into chlorophyll *a*. Synthesis of ALA represents the first committed step in

chlorophyll biosynthesis and serves as a key control point for regulating chlorophyll formation. δ -aminolevulinic acid dehydratase (ALAD) is an important enzyme of tetrapyrrole synthesis. Where, it catalyses the condensation of two δ -aminolevulinic acid molecules to form the pyrrole molecule, porphobilinogen. Reduction in chlorophyll and carotenoid, ALA content, as well as, ALA synthesizing activity, δ ALAD, and porphobilinogen deaminase (PBGD) activities by heavy metals, such as As, Hg and Cu have been reported in maize leaf segments (Jain and Gadre, 2004 a,b; Hirve and Jain, 2022). Therefore, there is a need to adapt some eco-friendly and cost-effective strategies like seed priming methods to alleviate the HM toxicity in plants. Magnetopriming is one of the non-invasive physical treatment that uses magnetic field (MF) pre-treatment to dry seeds before sowing to improve seed vigour and field emergence (Sarraf et al., 2020). The use of MF has gained attention in recent years as an alternative method for improving crop growth and yield under unfavourable environmental conditions (Sarraf et al., 2020, 2021; Ercan et al., 2022). Further, SMF pretreatment has been found to improve stress tolerance in plants exposed to cadmium (Chen et al., 2011; Vyas et al., 2024), drought (Baghel et al., 2018), UV-B (Kataria et al., 2021; Raipuria et al., 2021), and salinity (Kataria et al., 2022, 2023). Magnetopriming induced alleviation of toxic effects of arsenic, cadmium and mercury have been reported in

mungbeans and soybean seedlings (Chen et al., 2011; Fatima et al., 2024; Prajapati et al., 2023; Vyas et al., 2024). However, there is no information currently available on its ability to alleviate the adverse effects of As and Hg toxicity on chlorophyll biosynthesis enzymes in soybean seedlings. Soybean (*Glycine max* L.) is the most widely cultivated seed legume and it provides edible oil, protein concentrate for livestock feeding and a variety of industrial products. As and Hg toxicity have been shown to negatively impact soybean plants, leading to decreased seed germination, growth, root/shoot biomass, chlorophyll content, photosynthetic efficiency, and yield (Armendariz et al., 2016; Baig et al., 2018; Ahmed et al., 2021). Regrettably, soils of Madhya Pradesh are contaminated with many toxic metals including As, Hg, and Pb, etc. (Malik et al., 2013). Thus, the main objective of the present study was to analyse the effects of magnetopriming using static magnetic field (SMF) under heavy metal toxicity specifically As and Hg on chlorophyll and carotenoids content along with sensitive biomarkers, including activities of enzymes involved in early steps of chlorophyll biosynthesis in soybean seedlings.

MATERIAL AND METHODS

Plant material

For the present investigation, seeds of soybean (*Glycine max* (L.), variety JS-

9560 were procured from the Indian Institute of Soybean Research (ICAR-IISR), Indore, India.

Magnetic field treatment

Soybean seeds were magnetoprimed using an electromagnetic field generator fabricated by 'AETec' Academy of Embedded Technology, Delhi, India. The electromagnet has cylindrical pole pieces with a 9 cm diameter and 16 cm length, each wrapped with 3000 turns of copper coil having resistance of 16 Ohm. A DC power supply (0–85 V/10 A) with constantly variable output current was used to obtain required strength of magnetic field between the poles. Based on the previous experiments, soybean seeds were exposed to SMF of strength 200 mT for 1 h in a gap of 5 cm between the pole pieces in a cylindrical-shaped sample holder of 42 cm³ capacities, made from a non-magnetic cardboard container. During magnetic treatment temperature was kept constant at 25°C. The magnetic field strength between the poles was measured with Indium arsenate probe attached to a gauss meter. Unprimed seeds kept away from the magnetic field served as controls in the experiments.

Treatment for imposing heavy metal stress

The magnetoprimed (MP) and unprimed (UP) soybean seeds were surface sterilized with 0.01% HgCl₂ for 2 min, and then washed three-times with distilled water. Sterilized

seeds were then placed in Petri plates (15cm diameter) lined by moistened germination paper with 10 ml of different concentrations of sodium arsenate (Na arsenate, 0.0, 0.025, 0.075, and 0.1mM) and mercuric chloride (HgCl₂, 0.0, 0.05 and 0.1mM). These Petri plates were kept for 4 days in growth chamber under dark conditions at 25 ± 1°C. Afterwards, Petri plates were transferred to continuous light of about 40W m⁻² intensity at 25 ± 5°C temperature for 24 h. Seedlings were then used for analyses of dry weight of seedlings (biomass), pigments (chlorophylls and carotenoids content) and chlorophyll biosynthesis enzymes.

Chlorophylls and carotenoids

Chlorophylls and carotenoids were extracted in 80% acetone and their content was calculated using the equation of Lichtenthaler and Welburn (1983). Treated tissues (200mg) were extracted in 5.0 ml of 80% acetone. The homogenate was centrifuged at 5000rpm for 10 min. By adding 80% acetone supernatant obtained was made up to 5.0 ml and its absorbance was measured at 646, 663, and 470 nm using a double beam spectrophotometer (Shimadzu UV-1800) against 80% acetone as blank. The total chlorophyll and carotenoids content were calculated by using the following equations:-

$$\text{Total chlorophylls } (\mu\text{g ml}^{-1}) = (7.18 \times A_{663}) + (17.32 \times A_{646})$$

$$\text{Carotenoid } (\mu\text{g ml}^{-1}) = [1000 (A_{470}) - 3.27 (\text{chl}a) - 104 (\text{chl}b)]/229$$

ALA synthesizing activity

The ALA synthesizing activity was determined according to the method of Tewari and Tripathy (1998).

Extraction:- The seedlings (100mg) were incubated in phosphate buffer (50mM, pH 6.0) containing levulinic acid (60 mM) for 4h in light and dark. After rinsing with distilled water thoroughly, the seedlings were homogenized in mortar and pestle using 1.0 ml of sodium-acetate buffer (1.0 M, pH 4.6). The extract was centrifuged at 10,000 rpm for 10 min and the supernatant obtained was used to estimate ALA.

Estimation:- The reaction mixture consisting of 0.7 ml supernatant, 0.8 ml distilled water and 0.1 ml ethylacetoacetate was kept in a boiling water bath for 10 min. After cooling, equal volume of modified Ehrlich reagent* was added to the reaction mixture and the colored complex formed was measured at 553 nm and enzyme activity was expressed as nmoles ALA formed h⁻¹ g⁻¹ FW.

ALAD Activity

δ-Aminolevulinic acid dehydratase activity was assayed using modified Ehrlich reagent. (Mauzerall and Granick, 1956) according to the method of Jain and Gadre (2004a).

Extraction:- The seedlings material (100 mg) was extracted in 2.0 ml of 50 mM Tris-HCl buffer (pH 8.2) containing 10 mM DTT at 4°C. The extract was centrifuged at 15,000 rpm for 30 min in a cooling centrifuge 4°C using 'Remi C-24 Plus' and the

supernatant was used as the source of enzyme.

Estimation: -Reaction mixture was prepared by mixing 1.35 ml Tris-HCl buffer (50mM, pH 8.2) containing 10 mM DTT, 0.27 ml of 1mg ml⁻¹ ALA and 0.08 ml of 20mM MgCl₂. The reaction was started with addition of 1.0 of the enzyme. The mixture was then incubated at 37°C for 1h and the reaction was arrested by adding 0.3 ml of 3 M TCA. After centrifugation at 5,000 rpm for 10 min, the supernatant obtained was mixed with an equal volume of modified Ehrlich reagent *. After 15 min the absorbance was measured at 553 nm. A zero time control was prepared in a similar manner. One unit of enzyme activity was expressed as one nmole of prophobilnogen formed h⁻¹ g⁻¹ fresh weight.

Tolerance Index (TI)

To assess the effect of magnetopriming on tolerance towards Na arsenate and mercuric chloride toxicity in soybean seedlings, the tolerance index of seedlings was calculated. The tolerance index, based on seedling dry weight was calculated for seedlings from unprimed and magnetoprimed seeds grown in different concentrations of Na arsenate and mercuric chloride. Ten seedlings were randomly selected from each treatment (magnetoprimed and unprimed seeds germinated under different concentrations of Na arsenate and mercuric chloride in triplicates (n= 3). For dry weight determination, the seedlings were dried in an oven at

80 °C for 72 h and weighed. TI was determined using the formula (mean seedling dry weight in HMs / mean seedling dry weight in control) × 100 (Iqbal and Rahmati, 1992).

Statistical analysis

All data presented in this study represent the mean ±standard error derived from three independent experimental replicates. Data analyzed by the Student's t-test, ##p<0.05, ###p<0.01, ####p<0.001 denote significant difference between control and different concentrations of Na arsenate and mercuric chloride; *p<0.05, **p<0.01, ***p<0.001 denote significant difference magnetoprimed and unprimed seedlings grown in different concentrations of Na arsenate and mercuric chloride.

RESULTS

Effect of magnetopriming on soybean seedlings under As and Hg toxicity: -

Chlorophyll and carotenoids

In the seedlings from unprimed seeds, the estimation of chlorophyll content revealed that arsenic toxicity caused decrease in the chlorophyll content by 10% and 13% respectively at 0.075 and 0.10 mM Na arsenate (Table 1A); however, magnetopriming increase chlorophyll by 20% and 22% respectively. On the other hand, at all the level of Na arsenate toxicity, there was enhancement in carotenoids content in the seedlings from both magnetoprimed and unprimed seeds.

At 0.075 and 0.10 mM Na arsenate concentrations the extent of carotenoid content promotion in magnetoprimered seeds was 17% and 22%, respectively (Table 1A).

Analysis of chlorophyll content showed that HgCl_2 treatment also caused significant decrease of 24% to 28% in chlorophyll content respectively at 0.05 and 0.10 mM HgCl_2 (Table 1B) in seedlings from unprimed seeds, while at the same concentrations of Hg the increase of 40% and 17% respectively was observed in the chlorophyll content in seedlings from magnetoprimered seeds as compared to their unprimed ones (Table 1B). The increase in carotenoids was obtained in the seedlings from unprimed and magnetoprimered seeds at all the levels of Hg toxicity. In magnetoprimered seedlings the extent of promotion in carotenoid content was 38% and 44% as compared to their unprimed seedlings respectively at 0.05 and 0.10 mM HgCl_2 (Table 1B).

ALA synthesizing activity and ALAD activity

The ALA synthesizing activity of unprimed soybean seedlings was inhibited by 49% and 61%, respectively at 0.075 and 0.1 mM Na arsenate. However, magnetoprimering increased the activity at both the concentrations; the effect was greater (88% increase) at 0.10 mM concentration (Table 2A). When soybean seedlings were treated with

0.1 mM Na arsenate toxicity, ALAD activity in UP seedlings was reduced by 24%. In contrast, magnetoprimered seedlings demonstrated increased ALAD activity across all Na arsenate concentrations, with a maximum increase of 49% at 0.1 mM Na arsenate toxicity (Table 2A).

Supply of 0.05 and 0.1 mM HgCl_2 to unprimed soybean seedlings also inhibited the ALA synthesizing activity, however magnetoprimered enhanced the activity at both the concentrations and effect was more substantial (163% increase) at 0.05 mM HgCl_2 concentration (Table 2B). Treatment of soybean seedlings with HgCl_2 inhibited ALAD activity in unprimed seedlings, while enhancement in the activity was observed in magnetoprimered seedlings, more pronounced enhancement of 65% was observed at 0.05 mM HgCl_2 concentration (Table 2B).

The correlation analysis revealed significant R^2 values for unprimed and magnetoprimered seeds for photosynthetic pigments and Chl biosynthesis enzyme activities with increasing concentrations of HgCl_2 .

Tolerance Index

TI was 100% for control seedlings and it was decreased with an increase in Na arsenate and HgCl_2 toxicity (Fig. 1). The TI after treatment with 0.025, 0.075 and 0.10 mM Na arsenate was 67, 43 and 35%, respectively for soybean seedlings obtained from

unprimed seeds (Fig. 1); and magnetoprimed seedlings showed higher values of TI respectively at all of these Na arsenate concentrations i.e. 83, 68 and 59% (Fig.1). Similarly, TI value of 88, 83 and 79% were observed for unprimed seedlings and magnetoprimed seedlings showed 96,93 and 83% respectively for 0.03, .05 and 0.10 mM HgCl₂ toxicity (Fig.1).

DISCUSSION

Estimation of chlorophyll content is commonly used to assess the impact of several environmental factors, as it is indicated by both visible symptoms and photosynthetic plant productivity. Reduction in chlorophyll content is one of the major phytotoxic effects of arsenic and mercury. In the present investigation, photosynthetic pigments- total chlorophyll and carotenoids have been found to decrease with increasing concentrations of arsenic and mercury toxicity in soybean seedlings emerge from unprimed seeds (Table 1 A,B). These results were in line with some previous studies on a variety of seedlings and plants exposed to arsenic stress (Srivastava and Sharma, 2014; Ahmad et al., 2020; Beniwal et al., 2023). Similarly, supply of 0.01 to 1.00 mM Na arsenate to the greening maize leaf segments have also been shown to decrease the chlorophyll content (Jain and Gadre, 1997).

High level of Hg²⁺ is strongly phytotoxic to cells and can decrease

the chlorophyll content (Liu et al., 2010). Numerous investigations have demonstrated that, over an extended period of exposure to Hg decreased the amounts of chlorophyll and carotenoid (Mondal et al., 2015; Safari et al., 2019). Since the Hg⁺² induced stress damage the thylakoid membranes and substitute compounds of the chlorophyll metabolic pathway, that is responsible for Chl *a* and *b* content in plants (Singh et al. 2020).

The carotenoid content was significantly increased by Na arsenate and HgCl₂ supply and exhibits dose dependent response in unprimed soybean seedlings (Table 1B). Heavy metals can enhance carotenoid production depending on the metal type, concentration, and plant species (Sinha et al., 2003). In addition to their structural functions in photosynthetic reaction centers and antennae, carotenoids also scavenge ROS, protect the photosynthetic system from oxidative damage (Popova, 2017; Houri et al., 2020).

Further, it is observed that magnetopriming significantly increased the amount of both total chlorophyll and carotenoids in presence of As and Hg toxicity (Table 1A,B). Likewise, MF treatment increased the chlorophyll content in sugar beet leaves (Rochalska et al., 2005), chlorophyll and carotenoid content in soybean (Atak et al., 2003; Shine et al., 2011), barley (Ercan et al., 2022) and date palm (Dhawi et al., 2009). Our results endorse the findings of Kataria et al. (2017) and

Baghel et al. (2016,2018) where enhanced level of chlorophylls and carotenoids was demonstrated in soybean plants by magnetopriming under water, salt and UV-B stress. Chen et al. (2011) also reported increment in the amount of photosynthetic pigments by magnetopriming in mungbean seedlings under Cd toxicity. Abdolmaleki et al. (2007) reported higher carotenoid production after MF accumulation involved in plant defence.

Furthermore, increase in carotenoid content by magnetopriming, may also contribute in enhancing the chlorophyll biosynthesis/ promoting chlorophyll content as the protective role offered by them against oxidative stress caused by HM toxicity. Enhanced chlorophyll content in plants by magnetopriming due to increase in gibberellic acid that activates the accumulation of Mg^{2+} and K^+ for chlorophyll synthesis has been shown by several researchers (Reina and Pascual, 2001; Hassanpour and Ghanbarzadeh, 2023). Hence, it is possible that increased availability of Mg^{2+} in magnetoprimered soybean seedlings may be responsible for chlorophyll synthesis and thus enhanced chlorophyll content under toxicity of As and Hg (Table 1A,B).

Inhibition of the enzymes that catalyze chlorophyll biosynthesis or photosynthetic electron transport chain could be the cause of decrease in photosynthetic pigments under

stressful conditions (Jain et al., 2015). Inhibitory effect of arsenic on chlorophyll biosynthesis has been reported through depletion of substrates and thiol group modulation of $-SH$ dependent enzymes in maize leaf segments (Jain et al., 2015). Likewise, in the present study, Na arsenate toxicity decreased the ALA synthesizing and δ -ALAD activities in unprimed seedlings; these have been found to increase by magnetopriming (Table 2A). Similarly, metalloids such as, As and Se have been found to inhibit the activity of ALAD, a sulfhydryl-containing enzyme in greening maize leaf segments (Jain and Gadre, 1994, 2004a). Arsenic has a high affinity for sulfhydryl groups ($-SH$), leading to the formation of a complex with ALAD that prevents its proper functioning. This inhibition results in the accumulation of δ -aminolevulinic acid (Flora, 2009, Jain et al., 2015). Further, it has been proposed that the Mg atom in the chlorophyll tetrapyrrole ring may be replaced by trivalent As atom, which could hamper the chlorophyll-biosynthesis process (Zhang et al., 2021). Previous studies have shown inhibition of ALAD activity by Cr toxicity, leading to decrease in chlorophyll content due to impaired utilization of δ -aminolevulinic acid, a precursor in chlorophyll biosynthesis (Hayat et al., 2012; Hirve and Jain, 2019).

Our results demonstrate a concentration dependent decrease in ALA synthesizing and ALAD activity

by *in vivo* supply of 0.0–1.0 mM HgCl₂ in soybean seedlings from unprimed seeds (Table 2B). However, ALA synthesizing and ALAD activities were significantly increased by magnetopriming at all the concentrations of Hg toxicity used as compared to their unprimed ones (Table 2B). R² value of 0.906 and 0.901 between Hg treatment and ALA synthesizing and ALAD activity in magnetoprimed seedlings of soybean indicated a very good correlation (Table 2B).

Similarly, *in vivo* supply of HgCl₂ has been reported to inhibit ALAD activity in excised greening maize leaf segments (Jain and Gadre, 2004b; Gupta et al., 2013). Decreased ALAD activity was found to lower the chlorophyll content level in presence of Cr toxicity (Hayat et al., 2012; Hirve and Jain, 2019). Moreover, ALAD is a sulfhydryl-containing metalloenzyme that is very sensitive to metals and has been used as a biomarker for bio-monitoring of heavy metal effects in plant (Vanparys et al., 2008). The decrease in photosynthetic activity by mercury toxicity has been reported due to inhibition in the process of chlorophyll biosynthesis (Jain and Gadre, 2004b; Singh et al., 2023). It has been suggested that HM can target chlorophyll in any of three ways- by enhancing the activity of chlorophyllase enzyme (Latif et al., 2020); by causing oxidation/reduction of chlorophyll molecules by the HM toxicity induced ROS (Mordi et al., 2015); inhibition of chlorophyll biosynthesis as some of the HM such

as Hg, Pb and Cd, are able to replace the Mg in the porphyrin ring of the chlorophyll molecules (Gao et al., 2020; Sharma et al., 2019; Riyazuddin et al., 2022).

ALA formation is a rate limiting step in chlorophyll biosynthesis. Decreased ALA synthesizing and ALAD activity in soybean seedlings of unprimed seeds under As and Hg toxicity might be due to limited availability of precursors for ALA synthesis (Table 2A,B). The enhancement of ALA synthesizing as well as increase ALAD activity by magnetopriming indicates the increase in the rate of early steps of chlorophyll biosynthetic pathway which may be responsible for increasing the chlorophyll content.

Overall, the results revealed that toxicity caused by Na arsenate is more severe than HgCl₂. The tolerance index (TI) decreased with increasing Na arsenate and HgCl₂ concentrations in both unprimed and magnetoprimed seedlings, while magnetopriming consistently resulted in a higher TI (Fig.1). It indicates that magnetopriming mitigated the inhibitory effects of these heavy metals and enhanced seedling tolerance. In the same way, magnetopriming has been shown to increase TI in soybean seedlings under salt and cadmium stress (Kataria et al., 2021; Vyas et al., 2024).

In conclusion, chlorophyll biosynthesis is reduced by arsenic and mercury toxicity during seedling

development due to reduction of enzymes (ALAS and ALAD) activities involved in early steps of Chl biosynthetic pathway. Activation of ALA synthesizing and ALAD activity by magnetopriming under the conditions of As and Hg toxicity indicates the beneficial effect of seed pretreatment by SMF on chlorophyll biosynthesis, which may be responsible for enhancing the chlorophyll content in magnetoprimed soybean seedlings. To the best of our knowledge, this is the first study

demonstrating the promoting effect of magnetopriming on chlorophyll content by stimulating the early steps of chlorophyll biosynthesis in soybean seedlings under As and Hg toxicity. Thus by promoting dry weight of seedlings (biomass), total chlorophyll, carotenoids content and ALAS and ALAD activities, magnetopriming enhanced the tolerance towards As and Hg toxicity in soybean seedlings as evidenced by the improved tolerance index.

Table 1A. Effect of magnetopriming on total chlorophylls and carotenoids content in soybean seedlings under As toxicity.

Treatment	Total Chlorophylls ($\mu\text{g g}^{-1}$ FW)			Carotenoids ($\mu\text{g g}^{-1}$ FW)		
Na arsenate conc.(mM)	UP	MP	%Increase	UP	MP	%Increase
0	514 $\pm$ 66 (100)	652 $\pm$ 75*(100)	27	40 $\pm$ 4 (100)	52 $\pm$ 3 ^{ns} (100)	30
0.025	502 $\pm$ 97 ^{ns} (98)	580 $\pm$ 68*(89)	16	56 $\pm$ 2 [#] (140)	60 $\pm$ 5 ^{ns} (115)	7
0.075	463 $\pm$ 20 [#] (90)	579 $\pm$ 57*(89)	20	60 $\pm$ 4 [#] (150)	70 $\pm$ 2 ^{**} (135)	17
0.100	449 $\pm$ 14 [#] (87)	558 $\pm$ 82*(86)	22	64 $\pm$ 11 [#] (160)	78 $\pm$ 8 ^{**} (150)	22
R²	0.904	0.795		0.871	0.913	

Magnetoprimed and unprimed soybean seeds were treated with varying concentrations of sodium arsenate and grown in continuous dark for 4 days and then transferred to continuous white light for 24 h at 25 $\pm$ 1°C.

Values are mean $\pm$ SE and in parentheses the relative values representing difference between control and various concentration of Na arsenate are mentioned. ns = non-significant, [#]p<0.05; denote significant difference between control (–As) and different concentrations of Na arsenate (–As) used. ^{**}p<0.01, *p<0.05; denote significant difference between magnetoprimed and unprimed seedlings.

Table 1B. Effect of magnetopriming on total chlorophylls and carotenoids content in soybean seedlings under Hg toxicity.

Treatment		Total Chlorophylls ($\mu\text{g g}^{-1}$ FW)			Carotenoids ($\mu\text{g g}^{-1}$ FW)		
HgCl ₂ conc.(mM)	UP	MP	%Increase	UP	MP	%Increase	
0	516 $\pm$ 23(100)	564 $\pm$ 21 ^{ns} (100)	9	13 $\pm$ 7(100)	17 $\pm$ 2 ^{ns} (100)	31	
0.05	394 $\pm$ 10 ^{###} (76)	550 $\pm$ 13 ^{***} (98)	40	16 $\pm$ 2 ^{ns} (123)	22 $\pm$ 7 ^{ns} (131)	38	
0.1	373 $\pm$ 6 ^{###} (72)	437 $\pm$ 10 ^{***} (78)	17	20 $\pm$ 3 ^{ns} (160)	29 $\pm$ 16 ^{ns} (170)	44	
R²	0.857	0.831		0.993	0.992		

Magnetoprimed and unprimed soybean seeds were treated with varying concentrations of mercuric chloride and grown in continuous dark for 4 days and then transferred to continuous white light for 24 h at 25 $\pm$ 1°C.

Values are mean $\pm$ SE and in parentheses the relative values representing difference between control and various concentration of HgCl₂ are mentioned. ns= non-significant, ^{###}p<0.001; denote significant difference between control (–Hg) and different concentrations of mercuric chloride (+Hg). ^{***}p<0.001; denote significant difference between magnetoprimed and unprimed seedlings.

Table 2A. Effect of magnetopriming on ALA synthesizing activity and ALAD activity in soybean seedlings under As toxicity.

Treatment		ALA Synthesizing Activity (nmole ALA formed h ⁻¹ g ⁻¹ FW)			ALAD Activity (nmole PBG formed h ⁻¹ g ⁻¹ FW)		
Na arsenate conc.(mM)	UP	MP	%Increase	UP	MP	%Increase	
0	41 $\pm$ 27(100)	80 $\pm$ 27 ^{ns} (100)	95	346 $\pm$ 111(100)	526 $\pm$ 85 ^{ns} (100)	52	
0.025	39 $\pm$ 19 ^{ns} (95)	55 $\pm$ 15 ^{ns} (68)	41	299 $\pm$ 105 [#] (86)	417 $\pm$ 84 [*] (79)	39	
0.075	21 $\pm$ 2 [#] (51)	38 $\pm$ 7 ^{**} (47)	81	283 $\pm$ 13 [#] (82)	395 $\pm$ 93 ^{**} (75)	40	
0.1	16 $\pm$ 1 [#] (39)	30 $\pm$ 1 ^{**} (37)	88	263 $\pm$ 63 ^{###} (76)	393 $\pm$ 72 ^{**} (75)	49	
R²	0.928	0.997		0.972	0.818		

Magnetoprimed and unprimed soybean seeds were treated with varying concentrations of sodium arsenate and grown in continuous dark for 4 days and then transferred to continuous white light for 24 h at 25 $\pm$ 1°C.

Values are mean $\pm$ SE and in parentheses the relative values representing difference between control and various concentration of Na arsenate are mentioned. ns= non-significant, ^{###}p<0.01, [#]p<0.05; denote significant difference between control (–As) and different concentrations of Na arsenate (+As) used for treatment of soybean seeds. ^{**}p<0.01, ^{*}p<0.05; denote significant difference between magnetoprimed and unprimed seedlings.

Table 2B. Effect of magnetopriming on ALA synthesizing activity and ALAD activity in soybean seedlings under Hg toxicity.

Treatment	ALA Synthesizing Activity (nmole ALA formed h ⁻¹ g ⁻¹ FW)			ALAD Activity (nmole PBG formed h ⁻¹ g ⁻¹ FW)		
HgCl ₂ conc.(mM)	UP	MP	%Increase	UP	MP	%Increase
Control	43±30(100)	78±11 [#] (100)	81	108±4(100)	143±23 ^{ns} (100)	32
0.050	27±19 ^{ns} (63)	71±14 ^{ns} (94)	163	81±28 ^{ns} (75)	134±59 ^{**} (94)	65
0.10	23±14 [#] (53)	47±12 ^{**} (66)	104	78±10 ^{##} (72)	101±13 ^{**} (71)	29
R ²	0.892	0.906		0.931	0.901	

Magnetoprimed and unprimed soybean seeds were treated with varying concentrations of mercuric chloride and grown in continuous dark for 4 days and then transferred to continuous white light for 24 h at 25±1°C.

Values are mean ±SE and in parentheses the relative values representing difference between control and various concentration of HgCl₂ are mentioned. ns= non-significant, ^{##}p<0.01, [#]p<0.05; denote significant difference between control (–Hg) and different concentrations of mercuric chloride (+Hg). ^{**}p<0.01; denote significant difference between magnetoprimed and unprimed seedlings

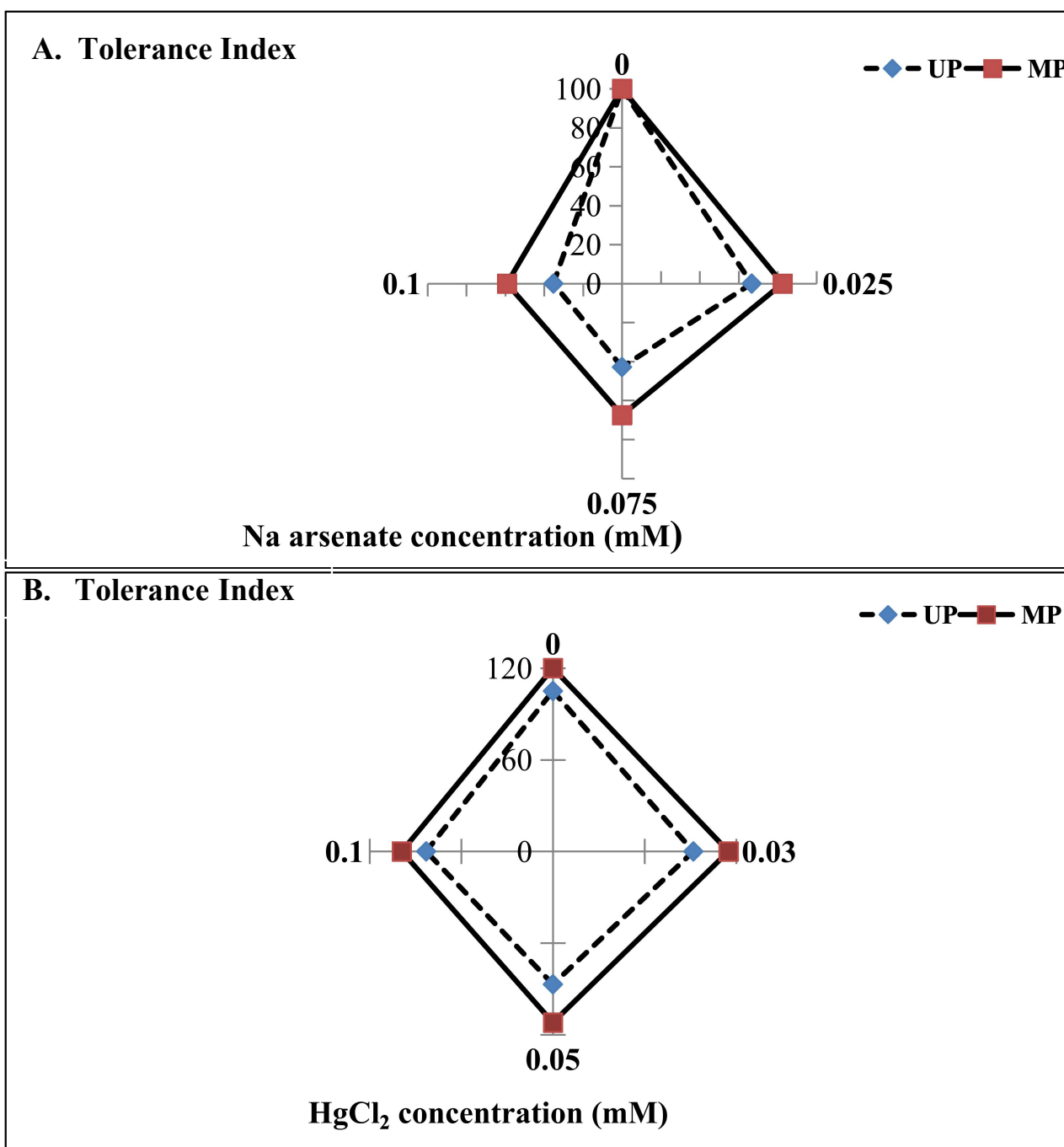


Fig.1. Effect of magnetopriming on tolerance index of soybean seedlings in terms of dry weight under As (A) and Hg (B) toxicity.

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Genetic Variability, Character Association and Path Analysis for Yield and its Components in Vegetable Soybean Genotypes (*Glycine max* [L.] Merrill)

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ABSTRACT

Fifty-five vegetable soybean genotypes were evaluated for character association and path analysis studies for thirteen yield and its contributing characters. Analysis of variance indicated that a significant amount of variation was present among the genotypes for different traits studied. Most of the characteristics in the present investigation shown considerable variability in plant height, branches per plant, number of pods per plant, number of pod clusters per plant, 100 seed weight at the R6 stage, 100 seed weight at maturity and green pod yield per plant. Green pod yield per plant is highly significant and positively correlated with plant height, followed by branches per plant, nodes per plant, days to 50 % flowering, days to maturity, number of pod clusters per plant, number of pods per plant, pod length and seeds per pod. Path analysis shows the positive direct effect on green pod yield per plant, 100-seed weight at maturity, number of pods per plant, number of nodes per plant, days to maturity, plant height and days to 50 % flowering. The results from the investigation suggested that days to maturity, plant height, branches per plant, number of pods per plant, number of pod clusters per plant, 100 seed weight at R6 stage and 100 seed weight at maturity, green pod yield per plant were important traits that should be taken into consideration as selection criteria for improving the green pod yield of the vegetable soybean.

Keywords: Correlation, Genetic advance, Heritability, Path coefficient analysis, Vegetable soybean.

Soybean (*Glycine max* [L.] Merrill)ⁱ is believed to have been domesticated from its wild relative *Glycine soja* in East Asia between 6,000 and 9,000 years ago (Carter *et al.*, 2004). It was grown in the Korean Peninsula and Central China by the first century. Soybean was introduced in Southeast Asia around the 15th to 16th century and in Europe before 1713. The

introduction of soybeans to the Indian subcontinent is believed to have occurred around 1000 AD, likely through the silk route from North Eastern India and the Himalayan mountains and consumed as a raw seed as a vegetable. The local names for vegetable soybean vary among Asian countries. However, 'Edamame'- the Japanese term is used worldwide for

vegetable soybean. Edamame is known as ‘Mau dou’ in China and ‘Poot kong’ in Korea (Mahoussi and Arnaud, 2020). The edamame has been cultivated for thousands of years in East Asia and is a popular snack in Japan, China, Thailand and Taiwan (Shanmugsundaram *et al.*, 1991). It is highly nutritious and rich in protein, cholesterol-free fat, fibre, iron, zinc, calcium, phosphorous, magnesium, potassium, isoflavones and tocopherol. Soybean has medicinal importance that reduces the risk of a range of hazardous diseases like atherosclerosis, osteoporosis and various types of cancer. Due to all of these advantages, vegetable soybeans have attracted people’s attention across the globe (Vineet Kumar *et al.*, 2006), hence bringing them to the notice of the diet and nutrition-conscious human community; their study is most important and need of an hour. There has been limited research conducted on the genetic aspects of vegetable soybean in India. The presence of genetic variability among genotypes is a prerequisite for improvement in crops. Heritability with high genetic advancement is important to understand the transfer of traits from one generation to another. Hence, the investigation was carried out to explore the performance of vegetable soybean genotypes for green pod yield and the important traits as vegetable.

MATERIALS AND METHODS

A field experiment was conducted at the experimental farm of MACS-Agharkar Research Institute, Pune (M.S.), India, situated at latitude 18°14' N, longitude 75°21' E and an altitude of 548.6 m above mean sea level. The experimental site received a total rainfall of 747.20 mm during the Kharif

2022-23 crop growth period. The average temperature ranged from 19.45 °C to 30.5 °C. A set of fifty-five soybean genotypes obtained from UAS, Bengaluru, AVRDC centre ICRISAT, Hyderabad and germplasm lines developed by the institute were used in the study. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Plot size was 4 x 1 m² with 45 cm x 7 cm spacing. The crop plot was initially supplied with FYM 5 t/ha during land preparation and supplemented with inorganic fertilizers at the rate 20:80:20:30 NPKS kg/ha during sowing as basal application. Zinc sulphate @ 25 kg/ha and Boron 10 kg/ha were also supplied to meet the micro-nutrient requirement. The crop was raised and maintained following the standard package of practices and harvested manually at the R6 stage (full pod stage) for green pod yield and at the physiological maturity stage for seed yield. The biometric observations on five randomly selected plants from each plot were recorded on days to 50 % flower, days to R6 stage, days to maturity, plant height (cm), number of nodes per plant, number of branches per plant, number of pods per plant, number of pod clusters per plant, pod length, number of seeds per pod, 100-seed weight at R6 stage (g), 100-seed weight at maturity (g) and green pod yield per plant (g) at R6 stage. Data analysis was done with R software version 4.3.2 and ‘Agri Analyze’ online software for different variability parameters, correlation and path coefficient.

RESULTS AND DISCUSSION

The ANOVA of the data on all growth and yield attributes was carried out, and the mean square of the characters studied is presented in Table 1. The results showed that the mean square was highly significant for all the thirteen characters studied. It indicates the presence of more significant exploitable variability for all

the traits ensures better scope for selecting these traits for future breeding work.

Genetic variability, Heritability and Genetic Advance in quantitative characters

Traits of the genotypes under the study were observed in a range (Table 2) viz., days to 50 % flowering from 27 to 43 days with mean value of 33, days to R6 stage from 60 to 82 days with mean of 69, plant height from 24 to 88 cm with mean of 45, number of nodes from 7 to 18 with mean of 9, number of pods per plant from 17 to 55 with mean of 29, number of pod clusters per plant from 9 to 25 with mean of 14, pod length from 3 to 6 cm with mean of 4.6, 100 seed weight at R6 stage from 14 to 95 with mean of 53, 100 seed weight at maturity from 7 to 40 with mean of 25, green pod yield per plant at R6 stage from 34 to 76 gm with mean of 50. The results showed a variation in the traits studied, which would be helpful in the variability study of the soybean genotypes in a broad sense and may help in an insightful understanding of these traits for further improvement.

Estimates of phenotypic and genotypic coefficient of variation, broad sense heritability, and genetic advance revealed significant variation ($P < 0.01$) for most traits. (Table 2). The highest GCV and PCV for plant height (38 and 38.53), branches per plant (29.53 and 34.35), number of pods per plant (29.87 and 32.63), numbers of pod clusters per plant (30.43 and 32.48), 100 seed weight at R6 stage (33.38 and 34.20), 100 seed weight at maturity (28.36 and 30.19) and green pod yield per plant (20.00 and 23.07).

The results are in agreement with Sonkamble *et al.* (2020). They observed high GCV and PCV for plant height, number of branches per plant, number of pods per plant, 100 seed weight at R6 stage, 100 seed weight at maturity and green pod yield per plant. Nayana and Fakrudin (2020) reported high GCV and PCV for plant height, branches per plant, number of pods per plant, number of pod clusters per plant, and 100 seed weight at maturity. Moderate GCV and PCV were recorded for nodes per plant (16.47 and 19.97), days to 50 % flowering (12.52 and 13.01) and pod length (12.88 and 14.37). Shilpashree *et al.* (2021) and Nayana and Fakrudin (2020) also reported moderate GCV and PCV for days to 50 % flowering and pod length, and Kuswantoro (2017) for nodes per plant. Whereas lower GCV and PCV were found for days to R6 stage (7.54 and 8.02) and days to maturity (5.74 and 6.31). Nayana and Fakrudin (2020) noticed the lowest GCV and PCV for days to the R6 stage and days to maturity. Lower difference between GCV and PCV for days to 50 % flowering, days to R6 stage and days to maturity indicate less influence of environmental factors on their expression and the chance of high selection gain, as reported. by Sonkamble *et al.* (2020) for days to 50 % flowering and days to maturity.

The estimate of broad sense heritability was highest for all the traits except seeds per pod, shown moderate heritability. Most of the traits exhibit high heritability coupled with high genetic advance as percentage of mean like days to 50 % flowering, plant height, number of nodes per plant, number of branches per plant,

number of pods per plant, number of pod clusters per plant, pod yield per plant, 100 seed weight at R6 stage and 100 seed weight at maturity. High heritability coupled with high genetic advance as a percentage of the mean is the best criterion for the phenotypic selection of these traits, and these traits appear to be controlled by an additive gene. Similar results were reported by Sonkambale *et al.* (2020) for days to 50 % flowering, plant height, number of branches per plant, number of pods per plant, green pod yield per plant, 100 fresh seed weight and 100 seed weight at maturity and Nayana and Fakrudin (2020) for a number of pods per plant and the number of pod clusters per plant. High heritability coupled with moderate genetic advance as a percentage of the mean was observed for days to the R6 stage and days to maturity. The results are in agreement with Sonkambale *et al.* (2020), who reported for days to maturity, and Shilpashree *et al.* (2021) for days to first harvest (R6). The traits seeds per pod had medium heritability and medium genetic advance as a percentage of the mean. Khumukcham *et al.* (2022) reported high heritability with low genetic advance as a percentage of the mean for the trait number of seeds per pod. Hence, the phenotypic selections for these traits might not be effective for crop improvement.

Character association and Path coefficient analysis

Genotypic and phenotypic correlations were calculated, followed by path coefficient analysis to partition the correlation coefficients of traits with

green pod yield per plant into direct and indirect effects. The genotypic correlation coefficient was seen to be higher than their respective phenotypic correlation coefficient, which is in agreement with Pawar *et al.* (2020) and Khanande *et al.* (2016).

In this experiment, (Table 3) green pod yield per plant is highly significant and positively correlated with plant height (0.5197) followed by branches per plant (0.4432), number of nodes per plant (0.4258), days to 50 % flowering (0.4108), days to maturity (0.3945), number of pod clusters per plant (0.3935), number of pods per plant (0.3873), pod length (0.3806) and seeds per pod (0.3523). Additionally, there is a positive but non-significant correlation with days to the R6 stage (0.256) followed by 100-seed weight at the maturity stage (0.246) and 100-seed weight at the R6 stage (0.194).

Similar results were reported by Akkamahadevi and Basavraj (2017) for plant height, number of branches per plant, number of pods per plant and 100 seed weight, Shilpashree *et al.* (2019) for days to 50 % flowering, number of pods per plant, plant height, Li *et al.* (2013) for number of seeds per pod and 100 seed weight at R6 stage and Sarutayophat (2012) for plant height, number of pods per plant in vegetable type of soybean genotypes. Pawar *et al.* (2020) for days to 50 % flowering, Lodhi *et al.* (2023) for days to maturity, Aditya *et al.* (2011) for number of nodes per plant and Baraskar *et al.* (2015) and Ekka and Lal (2016) for number of pod clusters per plant in grain type of soybean genotypes.

Path coefficient analysis is a valuable tool for understanding different traits' direct and indirect effects on pod yield per plant. In the present investigation (Table 4), it is seen that the residual effect of 47.065% indicates that independent variables included in the study are not sufficient to explain the 47.065% variation present in the dependent variable, green pod yield per plant; in other words, 52.935% variation in the dependent variable was explained by independent variables included in this experiment.

The positive direct effect on green pod yield per plant was shown by 100-seed weight at maturity (9.5214), number of pods per plant (2.3764), number of nodes per plant (1.60483), days to maturity (0.32425), plant height (0.16633) and days to 50 % flowering (0.1597). Similar observations were reported as a direct effect on green pod yield per plant by Shilpashree *et al.* (2019) for days to flowering, days to R6 stage and number of pods per plant, Akkamahadevi and Basavraj (2017) for plant height, number of pods per plant and 100 seed weight. Li *et al.* (2013) for pods per plant, Sarutayophat (2012) observed a number of pods per plant in vegetable types of soybean genotypes. Also, a direct effect on seed yield per plant was observed by Pawar *et al.* (2020) for the number of nodes per plant and Sulistyo *et al.* (2018) for days to maturity, plant height and nodes per plant in grain type of soybean genotypes.

The negative direct effect on green pod yield per plant was shown by 100-seed weight at the R6 stage (-6.2512), seeds per pod (-1.13572) and number of

branches per plant (-0.85093). Similar observations were reported by Sarutayophat (2012) for 100 seed weight at the R6 stage on green pod yield per plant and Barskar *et al.* (2015) for branches per plant and seeds per pod on seed yield per plant.

CONCLUSION

Most of the traits studied in the present investigation exhibited substantial variation for plant height, branches per plant, number of pods per plant, number of pod clusters per plant, 100 seed weight at R6 stage and 100 seed weight at maturity, green pod yield per plant shows high GCV, PCV, high heritability and high genetic advance as percentage of the mean. This indicates that there is additive gene action and scope for crop improvement through mass selection, progeny selection or any other suitable method for exploiting additive genetic variance. Almost all the traits show positive and significant correlation with green pod yield per plant except days to R6 stage, 100 seed weight at R6 stage and 100 seed weight at maturity shows positive and non-significant correlation. Thus, the selection for a high green pod yield in vegetable soybean should place maximum emphasis on these traits. Days to 50 % flowering, plant height, days to maturity, number of nodes per plant, number of pods per plant and 100 seed weight at maturity show a direct positive effect on green pod yield per plant. In contrast, a negative direct effect was shown by 100 seed weight at the R6 stage, seeds per pod and number of branches per plant.

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Table 1. Analysis of variance for traits studied in the vegetable soybean genotypes.

Sr. No.	Characters	Replication	Treatment	Error
1.	Days to 50 % flowering	3.00	53.12**	1.36
2.	Days to R6 stage	4.44	84.13**	3.49
3.	Days to maturity	7.64	85.72**	5.53
4.	Plant height (cm)	18.12	883.15**	8.07
5.	Number of nodes per plant	1.22	8.03**	1.09
6.	Number of branches per plant	0.39	2.01**	0.22
7.	Number of pods per plant	32.12	245.45**	14.84
8.	Number of clusters per plant	0.06	55.82**	2.48
9.	Pod length (cm)	0.27*	1.17**	0.09
10.	Seeds per pod	0.30	0.40**	0.16
11.	100 seed weight at R6 stage	35.81	950.86**	15.38
12.	100 seed weight at maturity	31.75*	158.15**	6.71
13.	Green pod yield per plant (gm)	37.57	336.85**	33.46

* and ** significant at $P < 0.05$ and $P < 0.01$, respectively

. Table 2. Estimates of variability parameters for different characters of vegetable soybean genotypes.

Sr. No.	Characters	Mean	Range	GCV (%)	PCV (%)	ECV (%)	h² (%)	GA	GA as per cent of mean (%)
1	Days to 50 % flowering	33	27-43	12.52	13.01	3.52	92.67	8.23	24.82
2	Days to R6 stage	69	60-82	7.54	8.02	2.72	88.5	10.04	14.62
3	Days to maturity	90	80-99	5.74	6.31	2.61	82.87	9.69	10.76
4	Plant height (cm)	45	24-88	38.00	38.53	6.31	97.31	34.70	77.22
5	Number of nodes per plant	9	7-18	16.47	19.97	11.29	68.22	2.58	27.98
6	Number of branches per plant	3	2-5	29.53	34.35	17.55	73.88	1.40	52.28
7	Number of pods per plant	29	17-55	29.87	32.63	13.12	83.82	16.53	56.33
8	Number of clusters per plant	14	9 -25	30.43	32.48	11.36	87.76	8.13	58.73
9	Pod length (cm)	4.65	3.3-6	12.88	14.37	6.35	80.42	1.10	23.8
10	seeds per pod	2.4	2-3	11.69	20.44	16.76	32.71	0.33	13.77
11	100 seed weight at R6 stage	52.89	14-95	33.38	34.20	7.41	95.3	35.51	67.14
12	100 seed weight at maturity	25.05	7-40	28.36	30.19	10.33	88.27	13.75	54.89
13	Green pod yield per plant (gm)	50.48	34-76	20.00	23.07	11.48	75.2	18.04	35.74

Table 3. Estimation of genotypic correlation coefficients for green pod yield and its component in vegetable soybean genotypes.

	DF	DR6	DM	PH	N	BR	PN	CL	PDL	SPP	G100SW	M100SW	GPY
DF	1												
DR6	0.5166 **	1											
DM	0.7501 **	0.7386 **	1										
PH	0.7796 **	0.2967 *	0.5838 **	1									
N	0.7047 **	0.345 **	0.6354 **	0.9002 **	1								
BR	0.8829 **	0.4001 **	0.6296 **	0.6941 **	0.6421 **	1							
PN	0.8041 **	0.3462 **	0.6023 **	0.733 **	0.7428 **	0.9136 **	1						
CL	0.8192 **	0.2985 *	0.551 **	0.7882 **	0.8652 **	0.9084 **	0.9168 **	1					
PDL	-0.3369 *	-0.2026 NS	-0.3109 *	-0.1624 NS	-0.3904 **	-0.4301 **	-0.5742 **	-0.4983 **	1				
SPP	0.3675 **	0.1729 NS	0.4331 **	0.4702 **	0.4354 **	0.2527 NS	0.2827 *	0.2926 *	-0.0483 NS	1			
G100SW	-0.4757 **	-0.2538 NS	-0.4422 **	-0.2813 *	-0.4955 **	-0.5066 **	-0.6457 **	-0.5544 **	0.8963 **	-0.1565 NS	1		
M100SW	-0.4105 **	-0.1434 NS	-0.3509 **	-0.2779 *	-0.4898 **	-0.4622 **	-0.6391 **	-0.553 **	0.9411 **	-0.0421 NS	0.9756 **	1	
GPY	0.4108 **	0.2558 NS	0.3945 **	0.5197 **	0.4258 **	0.4432 **	0.3873 **	0.3935 **	0.3806 **	0.3523 **	0.1943 NS	0.2459 NS	1

DF: days to 50 % flowering, DR6: days to R6 stage, DM: days to maturity, PH: plant height (cm), N: number of nodes per plant, BR: number of branches per plant, PN : number of pods per plant, CL: number of clusters per plant, PDL: pod length (cm), SPP: seeds per pod, G100SW:100 seed weight at R6 stage, M100SW:100 seed weight at maturity, GPY: green pod yield per plant (gm) * and ** significant at P<0.05 and P< 0.01, respectively.

Table 4. Path coefficient analysis for green pod yield and its component in vegetable soybean genotypes.

Trait	DF	DR6	DM	PH	N	BR	PN	CL	PDL	SPP	G100S W	M100S W	r _g	
DF	0.160	-0.614	0.243	0.130	1.131	-	0.751	1.911	-1.183	0.736	-0.417	2.974	-3.908	0.410 **
DR6	0.083	-1.188	0.240	0.049	0.554	-	0.340	0.823	-0.431	0.443	-0.196	1.587	-1.365	0.259 NS
DM	0.120	-0.878	0.324	0.097	1.020	-	0.536	1.431	-0.795	0.679	-0.492	2.764	-3.341	0.395 **
PH	0.124	-0.353	0.189	0.166	1.445	-	0.591	1.742	-1.138	0.355	-0.534	1.759	-2.646	0.520 **
N	0.113	-0.410	0.206	0.150	1.605	-	0.546	1.765	-1.249	0.853	-0.494	3.098	-4.663	0.426 **
BR	0.141	-0.475	0.204	0.115	1.030	-	0.851	2.171	-1.311	0.940	-0.287	3.167	-4.401	0.443 **
PN	0.128	-0.411	0.195	0.122	1.192	-	0.777	2.376	-1.324	1.255	-0.321	4.036	-6.085	0.387 **
CL	0.131	-0.355	0.179	0.131	1.388	-	0.773	2.179	-1.444	1.089	-0.332	3.466	-5.265	0.394 **
PDL	-	0.054	0.241	-0.101	-0.627	-	0.366	1.365	0.719	-2.185	0.055	-5.603	8.960	0.381 **
SPP	0.059	-0.205	0.140	0.078	0.699	-	0.215	0.672	-0.422	0.105	-1.136	0.978	-0.401	0.352 **
G100S	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W	0.076	0.302	-0.143	-0.047	-0.795	0.431	1.534	0.800	-1.958	0.178	-6.251	9.289	0.194 NS	
M100S	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W	0.066	0.170	-0.114	-0.046	-0.786	0.393	1.519	0.798	-2.056	0.048	-6.099	9.521	0.2466 NS	

DF: days to 50 % flowering, DR6: days to R6 stage, DM: days to maturity, PH: plant height (cm), N: number of nodes per plant, BR: number of branches per plant, PN: number of pods per plant, CL: number of clusters per plant, PDL: pod length (cm), SPP: seeds per pod, G100SW:100 seed weight at R6 stage, M100SW:100 seed weight at maturity. Diagonal values represent the direct effects, r = genotypic correlation, Residual effect = 47.065, * and ** significant at P<0.05 and P< 0.01, respectively.

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Genetic Variability for Yield and Components in Early Segregating Generation of Soybean (*Glycine max* (L.) Merrill)

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ABSTRACT

Soybean being a self-pollinated crop, available genetic variability is very low, and hence, there is a necessity to generate variability through recombination or resorting to mutation. In this regard, three crosses have been effected between widely adaptable (JS 335), rust resistant (DSb 21) and exotic line (EC 241780). Early segregating generation have been studied for various yield and yield components. The difference between the phenotypic coefficient of variability and the genotypic coefficient of variability is less for all the traits in all the three crosses, indicating the heritable nature of the traits. Segregating populations of all three crosses exhibited a wide range of variation for days to 50 % flowering, plant height, days to maturity, number of pods per plant, pod weight per plant, 100 seed weight, harvest index and seed yield per plant indicating scope for selection of these traits. The high genotypic and phenotypic coefficient of variation coupled with high heritability for number of pods per plant, pod weight per plant and seed yield per plant in all the three crosses suggesting higher genetic component governing these traits and hence amenable for selection based on phenotype.

Key words: Soybean, Heritability, Segregating generation, Genetic variability

Soybean (*Glycine max* (L.) Merrill) owes a worldwide reputation by virtue of its high-quality protein and low-cholesterol edible oil (Rizzo and Baroni, 2018). It has a higher protein content of 40 per cent and is a more protein-rich source than any of the common vegetable or animal food sources. Soybean oil has mainly five common fatty acids viz., 10 % palmitic acid (16:0), 4 % stearic (18:0) both of which are unsaturated, 20 % oleic (18:1) which is mono unsaturated, 55 % linoleic acid (18:2) and 10 % linolenic acid (18:3) which are poly unsaturated fatty acids

(Msanne *et al.*, 2020; Schmutz *et al.*, 2010). In view of its potentiality and wide range of agricultural, industrial and medicinal values, soybean is rightly described as “nature’s unique gift” to mankind. The area under soybean cultivation expanded significantly as a result of its nutritive, economic importance and diverse domestic usage.

Currently, soybean ranks first in the oilseed crop both in area and production in India (Basavaraja *et al.*, 2016). Over a decade, the area under soybean has increased consistently,

besides its spread to new areas all over India from 3000 ha in 1969 to 10.80 million ha during 2018-19. Soybean production increased from 2.49 million tonnes in 1991-92 to 12.10 million tonnes during 2018-19. This corresponds to a growth rate of 15-20 per cent per annum, which is obviously one of the highest for any crop in the recent past and resulted in the radical improvement of the rural economy (Basavaraja *et al.*, 2016).

Plant breeders always aim to increase production by utilizing available genetic resources. Soybean being a self-pollinated crop, the available genetic variability is less, and hence breeders resort to the generation of genetic variability either through recombination or inducing mutation. Induced mutation leads to random changes; recombination is the preferred means to generate genetic variability. In this regard, efforts have been made using the high-yielding potential genotypes from the existing germplasm, utilizing them in a hybridization programme, and isolation of superior segregants in the segregating population which is the preferred breeding strategy in highly self-pollinated crops like soybean. The success of selection depends on the magnitude of variation created through hybridization. The success in creation of genetic variation depends on the use of parents which are diverse in their traits of interest. In the present study, an effort is made to generate variability using a widely adaptable cultivar (JS 335), the first-ever rust-resistant cultivar (DSb 21) and an exotic collection with rust resistance (EC 241780) which will pave way for selection of superior genotypes.

MATERIALS AND METHODS

The material comprised of three second-generation populations derived using three parents *viz.*, DSb 21, JS 335 and EC 241780. Among these, JS 335 is a high-yielding and widely adoptable cultivar but susceptible to soybean rust, DSb 21 is a rust-resistant released cultivar having EC 241778 as the donor for rust resistance, and EC 241780 is a rust-resistant exotic collection (Basavaraja *et al.*, 2016). The salient features of these parents are given in Table 1. These three genotypes were crossed in possible combinations like susceptible x resistant (JS 335 x EC 241780), resistant x susceptible (EC 241780 x JS 335) and resistant x resistant (DSb 21 x EC 241780) during *summer* 2015. The F₁'s, along with parents in all three crosses, were raised during Kharif 2015. True F₁'s were selfed to advance to the second segregating generation.

The F₂ populations of three crosses *viz.*, JS 335 x EC 241780 consisting of 350 plants, EC 241780 x JS 335 with 456 plants and DSb 21 x EC 241780 consisting of 432 plants were sown with a spacing of 45 cm between the rows and 10 cm between the plants at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad (latitude-15.4589° N, and longitude-75.0077° E) during *Kharif* 2016. The observations were recorded for yield and its components *viz.*, days to flowering, plant height (cm), number of branches per plant, days to maturity, number of pods per plant, pod length (cm), pod weight per plant (g), number of seeds per pod, 100

seed weight (g), harvest index (%) and seed yield per plant (g) from individual plants in all the three crosses.

The mean and variance were assessed based on the formula given by Singh and Chaudhary (1977). The minimum and maximum values on the basis of individual plant observations were used to know the range of variation for a given trait. Genotypic and phenotypic variances and coefficients of variance were computed based on mean and variance calculated using the data of individual plants in all the three crosses. Heritability in the broad sense was computed as the ratio of genetic variance to the total phenotypic variance, as suggested by Hanson *et al.* (1956), and expressed as a percentage. The statistical analysis was performed employing INDOSTAT 9.1 software.

RESULTS AND DISCUSSION

A broad spectrum of variability is fundamental for the success of any plant breeding programme since it provides an opportunity for the plant breeder to use his skill and art in making useful selections. The coefficient of variation indicates only the extent of variability existing among the segregating generation of a cross for various traits but does not give any information about the heritable portion of it. Therefore, it is essential to know about heritability which permits the greater effectiveness of selection by separating out the environmental influence from the total variability. This indicates the accuracy

with which a genotype can be identified by its phenotypic performance.

The difference between the phenotypic coefficient of variation and genotypic coefficient of variation is less in all three the crosses for the various yield and yield contributing traits in the second generation (Tables 2, 3 and 4), indicating less influence of environment on the expression of these traits. Segregating populations of all three crosses exhibited a wide range of variation for days to flowering (31 to 48 days), plant height (15 to 77 cm), days to maturity (80 to 102 days), number of pods per plant (10 to 108), pod weight per plant (7.78 to 47.88 g), 100 seed weight (8.6 to 15.8 g) from individual plant, harvest index (30.47 to 65.62 %) and seed yield per plant (6.55 to 28.98 g). This variation indicated the scope for selection for these traits, and these genotypes may be allowed for attaining homozygosity. Seed yield per plant, number of pods per plant and pod weight per plant in all the three crosses, viz., JS 335 x EC 241780, EC 241780 x JS 335 and DSb 21 x EC 241780 had the highest PCV and GCV, indicating the existence of substantial variability for these traits. It also indicated greater scope for selection to improve these traits. Earlier, Sharma (1980), Chauhan and Singh (1982), Jagtap and Mehetre (1994) and Yadav (2006) reported higher phenotypic and genotypic coefficient of variation for plant height, pod length, number of pods per plant and seed yield per plant. However, moderate PCV and GCV were reported by Bangar *et al* (2003) for number of pods per plant. Such contrasting results are expected due to the

choice of different parents used in the hybridization program. In the present study, moderate PCV and GCV were noticed for plant height and number of branches per plant in all the three crosses, indicating less scope for selection for these traits. Moderate PCV and GCV were observed for 100 seed weight in the crosses JS 335 x EC 241780 and EC 241780 x JS 335, for harvest index in the cross EC 241780 x JS 335 and for pod length in the cross DSb 21 x EC 241780. Low PCV and GCV were noted for days to 50 % flowering, days to maturity and number of seeds per pod indicating no scope for selection for these traits in the studied material.

The estimates of heritability were high for plant height, number of branches per plant, number of pods per plant, pod length, pod weight per plant, 100 seed weight and seed yield per plant in all the three crosses indicating heritable component governing these traits. Earlier, high heritability was reported by Nirmalakumari and Balasubramanian (1993), Shrivastava and Shukla (1998), Singh and Singh (1999) and Agarwal *et al.* (2001) for seed yield per plant, Jagtap and Mehetre (1994), Taware *et al.* (1997) and Basavaraja (2002) for seeds per plant. Nirmalakumari and Balasubramanian (1993), Vimaladevi (1993) and Mahajan *et al.* (1994) for number of pods per plant while studying the genetic variability and selection indices for physiological and agronomic traits contributing to seed yield in soybean. High heritability was noted for harvest index in the crosses JS 335 x EC 241780 and DSb 21 x EC 241780. Srivastav and Jain (1994) and

Basavaraja (2002) also reported high heritability for harvest index while studying the variability and co-heritability estimates for physiological and economic attributes in soybean. Prediction of successful selection becomes more accurate if it is based on estimates of heritability coupled with genetic advance because it gives estimates not only of genetic contribution but of expected genetic gain out of the selection as well. High heritability coupled with high genetic advance as per cent of mean was observed for number of branches per plant, number of pods per plant and seed yield per plant (g), indicates the presence of additive gene action in the inheritance of these traits and improvement of these traits is possible through simple selection. Earlier, Hina Kausar (2005) also reported higher heritability accompanied with higher genetic advance for number of pods per plant while studying soybean genotypes. High heritability associated with medium genetic advance was observed for plant height and pod weight per plant in all the three crosses. This indicates that these characters are conditioned by both additive and non-additive gene actions. Hence, selection based on phenotypic observations alone may not be very effective for these traits. In summary, large genetic variability was created in F₂ generation using widely adopted cultivar and exotic lines for various traits in soybean. Further, high heritability coupled with high genetic advance for most of the yield components would indicate scope for selection of these traits based on phenotype.

Table 1. Salient features of the parents used in the study

Sl. No.	Variety /genotype	Pedigree	Duration (days)	Yield potential (q/ha)	Salient features
1.	JS 335	JS 78-77 x JS 71-05	85-90	25-30	Purple flowers; pubescence sparse or almost absent on stem ,leaves and pods; yellow seed coat, semi -determinate growth habit; tolerant to pod shattering up to 8-10 days after maturity and highly susceptible to rust.
2.	DSb 21	JS 335 x EC 241778	90-95	30-32	Purple flowers; pubescence - almost absent on stem, leaves and pods; yellow seed coat; semi-determinate growth habit; brown hilum; tolerant to pod shattering up to 8-10 days after maturity; highly resistant to rust.
3.	EC 241780	An exotic Germplasm line	110-120	Low Yielding	Purple flowers, pubescence-tawny on stem, leaves and pods, semi-determinate growth habit; yellow seed coat, brown hilum and highly resistant to rust.

Table 2. Genetic variability parameters for seed yield and its components in F₂ population of cross JS 335 x EC 241780

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h ² (bs)	GAM (%)
1	Days to initiation of flowering	40.42	36.00	44.00	3.88	1.74	20.14	1.61
2	Plant height (cm)	46.88	15.00	76.00	20.64	18.83	83.22	35.38
3	Number of branches per plant	5.03	2.00	6.00	21.20	19.60	85.42	37.31
4	Days to maturity	90.89	80.00	96.00	2.31	1.34	33.92	1.61
5	Number of pods per plant	61.00	19.00	108.00	32.64	32.16	97.08	65.29
6	Pod length (cm)	3.80	2.88	4.74	10.21	9.10	79.49	16.72
7	Pod weight per plant (g)	25.67	7.78	38.25	25.79	25.28	96.10	51.05
8	Number of seeds per pod	2.84	2.20	3.60	6.32	4.55	51.77	6.74
9	100 Seed weight (g)	12.52	8.6	15.70	12.98	12.42	91.56	24.49
10	Harvest index (%)	44.95	30.47	57.17	11.04	9.04	67.02	15.25
11	Seed yield per plant (g)	17.22	6.55	25.50	24.51	23.70	93.48	47.20

Table 3. Genetic variability parameters for seed yield and its components in F₂ population of cross EC 241780 x JS 335

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h ² (bs)	GAM (%)
1	Days to initiation of flowering	38.39	31.00	46.00	6.41	4.35	46.09	6.09
2	Plant height (cm)	48.43	22.00	76.00	20.23	18.41	82.84	34.52
3	Number of branches per plant	4.61	2.00	6.00	22.11	18.79	72.22	32.90
4	Days to maturity	90.06	83.00	96.00	2.13	1.52	50.93	2.24
5	Number of pods per plant	57.43	15.00	98.00	30.75	30.16	96.21	60.94
6	Pod length (cm)	3.73	2.30	4.60	8.84	7.47	71.30	12.99
7	Pod weight per plant (g)	27.12	14.85	46.37	28.97	28.27	95.22	56.82
8	Number of seeds per pod	2.78	2.20	3.00	4.94	3.35	45.94	4.68
9	100 Seed weight (g)	11.95	9.10	15.80	12.36	11.80	91.13	23.20
10	Harvest index (%)	43.10	31.02	65.62	14.40	11.00	58.28	17.29
11	Seed yield per plant (g)	16.95	9.28	28.98	28.97	28.02	93.57	55.83

Table 4. Genetic variability parameters for seed yield and its components in F₂ population of cross DSb 21 x EC 241780

Sl. No.	Traits	Mean	Minimum	Maximum	PCV (%)	GCV (%)	h² (bs)	GAM (%)
1	Days to initiation of flowering	41.73	34.00	48.00	5.26	2.44	21.48	2.33
2	Plant height (cm)	52.50	18.00	77.00	16.30	14.92	83.80	28.14
3	Number of branches per plant	4.65	2.00	6.00	23.30	19.75	71.84	34.49
4	Days to maturity	92.71	88.00	102.00	2.69	1.98	54.32	3.01
5	Number of pods per plant	59.99	10.00	99.00	32.43	31.67	95.38	63.72
6	Pod length (cm)	3.72	2.80	4.70	11.43	10.23	80.11	18.87
7	Pod weight per plant (g)	28.61	12.23	47.88	28.51	27.94	96.08	56.42
8	Number of seeds per pod	2.78	2.20	3.40	5.92	4.43	55.82	6.81
9	100 Seed weight (g)	12.12	10.00	15.60	10.35	9.69	87.67	18.69
10	Harvest index (%)	44.03	31.20	61.94	11.34	9.12	64.76	15.12
11	Seed yield per plant (g)	17.88	7.64	27.93	28.38	27.61	94.70	55.35

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Evaluation of Indian Soybean Genotypes for *Agrobacterium*-Mediated Transformation Efficiency using half seeds

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ABSTRACT

To see the effect of genotypes on Agrobacterium-mediated transformation using half-seed explants, 83 soybean genotypes were selected, and co-cultivated for 5 d in dark with Agrobacterium tumefaciens strain EHA105 carrying the binary vector pCAMBIA1305.1 encoding the hptII and GUSPlus genes. We observed differential genotypic variation of susceptibility in soybean towards Agrobacterium-tumefaciens infection. Based on the rate of transient GUS expression, these genotypes were categorized as highly, moderately, and weakly susceptible. Five genotypes, namely, KHSb2; Pusa-16; DS 228; NRC 149, and JS 72-280, were found to be highly susceptible showing >70% transient GUS expression whereas 30 genotypes were moderately susceptible (30–70%); and 48 genotypes showed very low transient GUS expression (<30%) and were categorized as weakly susceptible. The analysis revealed that different soybean genotypes have variable susceptibility to Agrobacterium-tumefaciens infection. The finding can lead to identification of susceptible soybean genotypes which could be used for successful transformation protocol.

Keyword: Half seeds, Genotypic variability, Transformation, Agro-infection, Transformation efficiency

Soybeans is widely grown for vegetable oil and human protein and used in food, feed, biofuel, and several other industrial and consumer applications (Chen et al., 2012). However, traditional plant breeding has significantly increased soybean productivity but due to the narrow genetic diversity and small gene pools of soybean germplasms, using conventional breeding techniques to achieve further breakthroughs has proven challenging (Vieira and Chen, 2021). New breeding technologies are

now necessary to encourage sustainable production and satisfy the growing demand for soybeans. Genetic modification (GM) using transgenes and, more recently, genome editing (GE) are two significant developments in soybean breeding. In both GM and GE investigations, effective transformation mechanisms are crucial (Gao, 2021). *Agrobacterium tumefaciens*-mediated transformation and microprojectile bombardment-mediated transformation are the two techniques for plant

transformation that are most frequently used. Both techniques have been developed and improved over the past 20 years, but *Agrobacterium tumefaciens*-mediated transformation is the preferred technique because it is less expensive, simple to use, and results in fewer transgenic copies with more stable gene expression. Since *Agrobacterium*-mediated transformation utilizing cot-node explants produced the first transgenic soybean plant, this technique has been improved, and the transformation efficiency varies between 2% and 10%, which is still quite low. Numerous studies have been conducted to increase the efficiency of transformation (Yan *et al.*, 2000; Ko and Korban, 2004; Olhoft *et al.*, 2007; Wang and Xu, 2008; Liu *et al.*, 2008; Shukla *et al.*, 2020; Paes de Melo *et al.*, 2020) but the transformation efficiency is still not sufficient to get enough transformants.

Soybean transformation is genotype-dependent. Since the susceptibility of soybean genotypes to *Agrobacterium* infection is a critical factor in the high degree of genetic transformation efficiency, the lack of highly effective genotypes susceptible to transformation is one of the main challenges in the development of transgenic soybean. Genotypic dependency restricted the application of this method in the routine production of elite and commercially valuable soybean cultivars as it's a time-consuming process for the transfer of transgene in commercial genotypes. If high transformation efficient genotypes are available in elite and commercially

valuable cultivars, the success rate of transgenic soybean production would be improved considerably (Jia *et al.*, 2015; Rani *et al.*, 2012). The availability of soybean genotypes with high transformation efficiency will help in studying the function of gene response for various metabolic and physiological processes of the soybean plant. Therefore, screening soybean genotypes from the available germplasm resources suitable to *Agrobacterium*-mediated genetic transformation has become the focus for optimizing the soybean transformation system and improving transformation efficiency that offers a significant advancement for soybean breeding programs allowing the production of novel and genetically diverse plant materials (Song *et al.*, 2013).

MATERIALS AND METHODS

Plant Materials: A total of 83 diverse Indian soybean cultivars were tested for genotypic variability using half seeds (Table 1).

Seed Sterilization: Healthy and bold seeds were surface-sterilized by exposure to chlorine gas. The chlorine gas was prepared using a mixture of 5 mL HCl (39.6%) and 100 mL sodium hypochlorite (4%) and kept for 16-18 h (Liu and Wei, 2002).

***Agrobacterium* Strain and Vector:** *Agrobacterium tumefaciens* strain EHA105 containing binary vector pCAMBIA1305.1 (CAMBIA, Australia) was used for genetic transformation. pCAMBIA1305.1

(<https://www.markergene.com/pcambia-vectors>) contained *hpt* as the plant selectable marker, kanamycin resistance gene as the bacterial selection marker, and an intron-containing *GUSPlus* gene from *Staphylococcus* as the reporter gene. *Agrobacterium tumefaciens* strain EHA105 was transformed with binary vector pCAMBIA1305.1 by preparing heat-shock Agro-competent cells of strain EHA105 following the protocol of Höfgen and Willmitzer (1988). The presence of pCAMBIA1305.1 plasmid was confirmed in the antibiotic-resistant bacterial colonies by colony PCR using 35 S promoter, and *nptII* primers.

***Agrobacterium* Culture and Infection**

Medium: *Agrobacterium* strain EHA105 harboring pCAMBIA1305.1 was cultured on Luria agar (LA) media (10 g/L casein enzymic hydrolysate, 5 g/L yeast extract, and 10 g/L NaCl, 1.5% agar; Himedia, India) containing 50 mg/L kanamycin and rifampicin each for 2-d at 28°C. A single colony of *Agrobacterium* was obtained from the plate and inoculated for 6 h at 28°C in 200 rpm into 50 ml Luria broth (LB, liquid LA) containing 50 mg/L of both kanamycin and rifampicin (primary culture). Subsequently, 500 µl of the 50 ml primary culture was mixed in a 200 ml LB culture and grown overnight at 28°C at 200 rpm using a shaker incubator. On the day of infection, bacterial pellet was obtained by centrifuging the overnight culture at 4000 rpm for 10 min and resuspended in infection medium containing 1/10 Gamborg's B5 medium (Gamborg *et al.*, 1968) supplemented with 2.3 g/L 2-[N-

Morpholino] ethane sulfonic acid (MES), 3% (30 g/L) sucrose (pH 5.4), filter sterilized 1.67 mg/L 6-BA, 0.25 mg/L GA3, and 50 mg/L AS. Bacterial cell density was adjusted to 0.7 OD600 using a nanodrop spectrophotometer (Denovix) before infection of explants and incubated at room temperature for 30 min before infection.

Explant Preparation: The sterilized seeds of taken cultivars were soaked in autoclaved distilled water overnight in the dark at 24°C. The half seed explants were prepared by using a scalpel to cut the imbibed seeds longitudinally along the hilum, then scraping off the seed coats (Paz *et al.*, 2006).

Infection and Co-cultivation: Half seed explants were immersed in *Agrobacterium tumefaciens* EHA105 suspension (0.7 OD600) harboring the binary vector pCAMBIA1305.1 for 10 min, at room temperature. After inoculation, 10-15 explants (apical regions directed upwards) were placed in the sterile petri dish (90 mm) containing semi-solid CCM, which is composed of infection medium additionally supplemented with 0.06% agar-agar (Himedia, India), filter sterilized cysteine (200 mg/L) and DTT (154.2 mg/L) with a piece of Whatman filter paper and then incubated at 24 °C in dark for 5-d.

Histochemical GUS Assay: After 5-d of co-cultivation, a histochemical GUS assay was performed following the method of Jefferson *et al.* (1987). Transformed half seed explants were incubated overnight at 37°C in GUS

solution. Following incubation, tissues underwent multiple washes with 70% ethanol until all of the chlorophyll was removed. The tissues showing blue color after the removal of chlorophyll was scored and GUS-stained transformed embryonic tips were counted as GUS-positive transformants.

Statistical Analysis: Transient GUS expression (%) in soybean cultivars was determined in three biological replications and each biological replication was measured in triplicate, the means and standard errors were calculated. The data were statistically assessed using the one-way ANOVA. The comparison of the variation between means was performed via Fisher's least significant difference (LSD) value through DMRT at a significance level of $p < 0.05$.

RESULTS & DISCUSSION

Host plant genotype significantly affects the level of *Agrobacterium* infection (Karami 2008). A variety of explants like cot-node, half seed, and embryonic tip have been used in the development of transgenic soybean (Chen *et al.*, 2018; Liu *et al.*, 2004; Olhoft *et al.*, 2003; Paz *et al.*, 2006; Rani *et al.*, 2012; Verma, 2015). We used half seeds of cultivated Indian cultivars for genotypic variability for transformation efficiency. In this study, *Agrobacterium*-mediated transformation efficiency in soybean was improved by selecting a highly susceptible soybean genotype. To see the effect of genotype on transformation using half seed explants, 83 soybean genotypes were taken for the study

(Table 1). Significant genotype variability for susceptibility to *Agrobacterium* infection was noted in Indian soybean genotypes. We observed that half seeds of different genotypes have different susceptibilities to agro-infection, and on the basis of the rate of transient GUS expression, these were categorized as highly (>70%), moderately (30-70%), and weakly susceptible (<30%) genotypes (Figure 1). Five genotypes, namely, KHSb2; Pusa-16; DS 228; NRC 149, and JS 72-280, were found to be highly susceptible and showed >70% transient GUS expression; 30 genotypes were moderately (30–70%) susceptible; and 48 showed very little (30%) transient GUS expression and were categorized as weakly susceptible. The result showed that the rate of transient GUS expression was significantly ($p < 0.05$, DMRT) higher in KHSb2 (95.3%); Pusa-16 (87.4%); DS 228 (86.2%); NRC 149 (78%), and JS 72–280 (71.3%) than in other genotypes. Higher *Agro*-susceptibility was also observed in DS 228 (100%), KHSb2 (96%), NRC 149 (96%), and JS 72-280 (92%) when embryonic tip explants were used as an explant in previous study (Shukla *et al.*, 2020) confirming that these 4 genotypes has some genetic factors which are responsible for higher transformation efficiency. Though these factors were not analyzed till date, a mapping population can be developed from a cross of highly and weakly susceptible genotypes to study the genetic factors responsible for the difference and map the genomic region regulating susceptibility to *Agrobacterium*

infection. Identified highly susceptible genotypes to Agro-infection in this study may improve the success rate of the development of transgenic soybean and once the agencies authorized for the distribution of transgenic crops in India have given their legal approval, transgenics produced from these cultivars can be released immediately. Moreover, these genotypes can also be directly utilized to investigate the function of genes with unknown roles in

order to enhance soybean performance under a variety of biotic and abiotic stress condition through the application of CRISPR-Cas9 gene editing or virus-induced gene silencing (VIGS) approaches.

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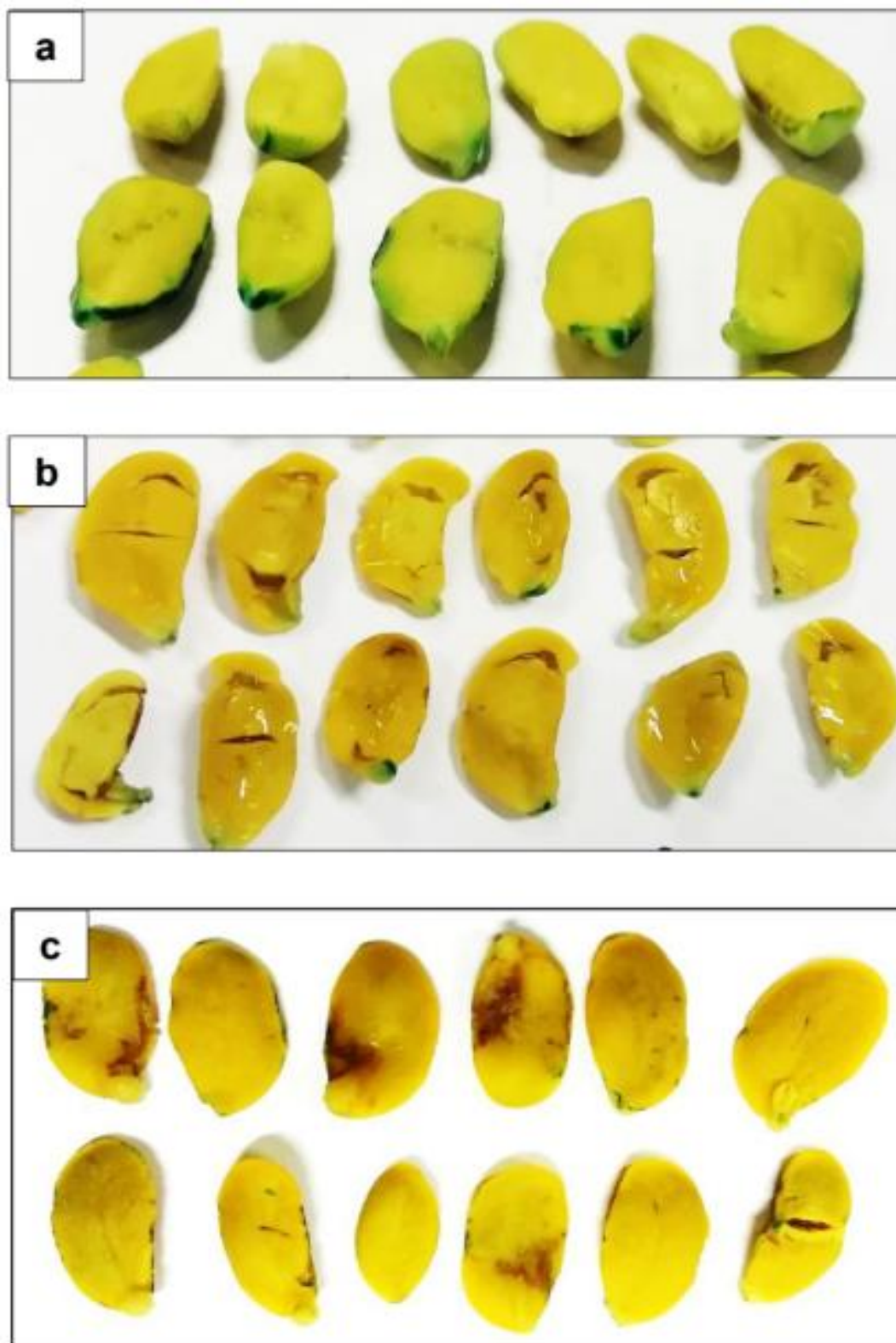


Figure 1: Genotypic effect on transient GUS expression of half seed explants (a) Highly susceptible genotype: JS 72-280 (b) Moderately susceptible genotype: MACS 124, and (c) weakly susceptible genotype PK 327

Table 1 Evaluation of genotypic variability of Indian Soybean genotypes for *Agrobacterium*-mediated transformation efficiency using half seeds.

No.	Genotype	TE (%)	No.	Genotype	TE (%)	No.	Genotype	TE (%)
1	KHSb2	95.3±0.7 ^a	29	JS 72-44	35.7±2.0 ^{op}	57	MAUS-1	16.1±0.9 ^{AB}
2	Pusa 16	87.4±1.7 ^b	30	NRC138	36.0±0.9 ^{op}	58	JS 79-81	13.2±0.8 ^{BC}
3	DS-228	86.2±2.9 ^b	31	JS95-60	35.4±0.7 ^{op}	59	Pusa 20	11.5±0.5 ^{CD}
4	NRC 149	78±2.2 ^c	32	Punjab-1	35.3±0.5 ^{op}	60	Monetta	11.5±0.6 ^{CD}
5	JS 72-280	71.3±0.6 ^d	33	PK 308	34.7±1.3 ^{op}	61	Kb-79	11.4±0.9 ^{CD}
6	Co 3	68.5±1.0 ^d	34	Tams-38	32.6±0.6 ^{pq}	62	PK 471	10.8±0.3
7	JS 80-21	65.1±0.3 ^{de}	35	NRC107	31.6±1.5 ^{pqr}	63	Type49	10.8±0.9 ^{CDE}
8	SL295	63.4±0.8 ^{ef}	36	Indira Soya	29.9±1.1 ^{qrs}	64	MAUS-32	8±2.9 ^{qrstu}
9	Alankar	60.0±1.0 ^{fg}	37	MACS-57	29.3±0.5 ^{qrst}	65	PK 327	8.5±0.6 ^{DEF}
10	MAUS-57	59.1±1.3 ^{fg}	38	PK 472	28.6±0.9 ^{qrstu}	66	SL525	7.3±0.4 ^{DEFG}
11	MACS-124	57.4±1.5 ^g	39	MAUS-32	28.1±2.9 ^{qrstu}	67	VLS47	6.6±0.5 ^{EFGH}
12	JS 335	56.5±1.7 ^g	40	PS 1225	28.2±1.3 ^{qrstu}	68	NRC 2	5.9±0.5 ^{FGHI}
13	PK 1042	51.7±0.85 ^h	41	JS 76-205	27.1±2.2 ^{rstu}	69	Pratap Soya 2	5.7±0.5 ^{FGHIJ}
14	MAUS-2	49.5±1.2 ^{hi}	42	SL-96	26.4±1.0 ^{stuvw}	70	MAUS-61	5.3±0.5 ^{FGHIJ}
15	Lee	48.9±0.9 ^{hij}	43	JS 2029	26.4±1.0 ^{stuvw}	71	JS 71-05	3.7±0.3 ^{GHIJK}
16	MAUS61-2	48.5±0.7 ^{hijk}	44	VLS1	25.1±2.1 ^{tuvwxy}	72	ADT-1	3.2±0.6 ^{GHIJK}
17	PK 564	47.3±1.8 ^{hijk}	45	Pusa 37	24.0±0.2 ^{uvwxy}	73	NRC109	3.2±0.4 ^{GHIJK}
18	JS97-52	46.8±1.1 ^{ijkl}	46	MAUS-158	23.3±0.6 ^{vwxy}	74	SL688	2.8±0.4 ^{GHIJK}
19	MAUS-81	46.2±1.5 ^{ijkl}	47	JS93-05	23.4±1.4 ^{vwxy}	75	PS 1347	2.7±0.5 ^{GHIJK}
20	JS 75-46	44.4±1.0 ^{klm}	48	Hardee	23.1±1.2 ^{vwxy}	76	Davis	2.6±0.5 ^{HJK}
21	JS 2	43.9±1.0 ^{klm}	49	NRC127	22.6±1.1 ^{wxy}	77	NRC142	2.9±0.4 ^{IJK}
22	Pusa 22	42.5±0.8 ^{lm}	50	Pusa 40	21.9±1.4 ^{vzA}	78	PS 1241	1.8±1.1 ^{IJK}
23	Ankur	41.6±0.7 ^m	51	PK 1029	20.2±0.9 ^{vzA}	79	JS 2034	1.5±0.4 ^{IJK}
24	Bragg	41.3±1.0 ^{mn}	52	Co 2	19.8±1.4 ^{vzA}	80	JS 90-41	1.3±0.5 ^{JK}
25	Improved Pelican	41.0±1.2 ^{mn}	53	NRC 37	19.2±1.3	81	NRC 12	0.0±0.0 ^K
26	MAUS-47	41.0±1.3 ^{mn}	54	PK 262	17.9±0.2 ^{zA}	82	PK 1092	0.0±0.0 ^K
27	RKS-24	39.9±1.3 ^{mno}	55	PRS1	17.4±0.8 ^{zAB}	83	VLS2	0.0±0.0 ^K
28	Gujarati Soya-2	35.6±1.5 ^{nop}	56	Lsb1	17.3±0.8 ^{zAB}			

*The data presented here are the average values of three replicates ± SD. Values followed with dissimilar letters differ significantly at p = 0.05 in accordance with LSD and DMRT. The transient GUS expression was calculated as follows: Transient GUS expression (in % = The number of positive transformants/the total infected explants × 100. TE=Transient Expression

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Efficacy of herbicides on growth, yield and quality of soybean under weed control treatments

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ABSTRACT

A field experiment entitled “Efficacy of Herbicides for Weed Control in Soybean [Glycine max (L.) Merrill]” was conducted during kharif 2023 at Agricultural Research Station, Ummedganj, Kota. The experiment comprised ten treatments viz., pendimethalin 30% EC + imazethapyr 2% SL @ 1.0 kg a.i./ha (PE) (RM), pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence, pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence + one hand weeding at 40 DAS, diclosulam @ 26 g a.i./ha as pre-emergence, diclosulam @ 26 g a.i./ha at pre-emergence + one hand weeding at 40 DAS, imazethapyr 10% SL @ 100 g a.i./ha (PoE) at 15 -20 DAS, sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix)@ 165 +80 g a.i./ha at 20 DAS, propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g a.i./ha at 20-25DAS (RM), two hand weeding at 20 & 40 DAS and weedy check was laid out in randomized block design with three replications.

The maximum values of growth parameters and yield attributes were obtained with two hand weeding, which were significantly superior over rest of the herbicides and weedy check except diclosulam @ 26 g a.i./ha as pre-emergence, diclosulam @ 26 g a.i./ha at pre-emergence + one hand weeding at 40 DAS with respect to some characters. Among the herbicidal treatments, application of pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence + one hand weeding at 40 DAS recorded maximum crop dry matter accumulation/plant, plant height and pods/plant which were significantly higher over weedy check.

All the weed management practices significantly enhanced seed and straw yields and quality (oil yield and protein yield) and nutrient uptake by soybean over weedy check. Two hand weeding at 20 and 40 DAS was registered highest soybean seed yield (2005 kg/ha), straw yield (2854 kg/ha), oil yield (412.19 kg/ha) and protein yield (843.52 kg/ha) followed by application of diclosulam @ 26 g a.i./ha at pre-emergence + one hand weeding at 40 DAS (1783, 2558, 359.85 and 736.52 kg/ha, respectively), which were significantly superior over weedy check.

Keywords: growth, yield, quality, herbicides, weed

In India, oilseed crops constitute the second-largest agricultural produce next to grains and are a vital component of the national economy. The oil extracted from oilseed crops plays a crucial role in the human diet and has numerous industrial, medical, and therapeutic applications. Soybean (*Glycine max* L. Merrill) is a major pulse and oilseed crop in India. On the world basis, during the year 2023-24 soybean occupied an area of 139.42 million ha with production is 396.95 MT and productivity is 2.85 MT/ha, respectively, On the national basis, during the year 2022-23 soybean occupied an area of 13.0 million ha with production is 11.88 MT and productivity is 0.91 MT/ha (U.S.D.A. 2024), Rajasthan occupied an area of 1.03 million ha and production is 0.99 million tonnes respectively (Anonymous 2022-23).

Soybean is a significant source of high-quality protein and edible vegetable oil, crucial for both human and animal nutrition. It contains approximately 40% quality protein, 23% carbohydrates, and 20% cholesterol-free oil. While herbicides provide effective and timely weed control, their application can be costly for the average farmer. Reducing herbicide costs and employing a well-planned tank mix of pesticides along with cultural weed control methods can benefit the crop by enhancing aeration and conserving moisture.

Weed Management is gaining popularity worldwide as a method of weed control, particularly in response to the increasing incidence of herbicide-resistant weeds. Weed management employs a combination of approaches to

manage weeds throughout the growing season. It leverages knowledge of weed biology (such as emergence, growth rate, and fecundity) and integrates multiple weed control tools to strategically target different stages of the weed life cycle, thereby inhibiting their growth and development with these considerations in mind, the present investigation was undertaken to study the experiment entitled “Efficacy of Herbicides for Weed Control in Soybean [*Glycine max* (L.) Merrill]”

MATERIAL & METHODS

A field experiment was conducted during *Kharif* season, 2021 at Experimental Farm, Department of Agronomy Latur, to study “Efficacy of Herbicides for Weed Control in Soybean [*Glycine max* (L.) Merrill]”. The soil was clayey in texture, low in available nitrogen (292.35 kg/ha), medium in available phosphorus (21.4 kg/ha), very high in available potassium (303.01 kg/ha) and slightly alkaline (pH 7.8) nature. The experiment comprised ten treatments viz., pendimethalin 30% EC + imazethapyr 2% SL @ 1.0 kg *a.i.*/ha (PE) (RM), pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence, pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence + one hand weeding at 40 DAS, diclosulam @ 26 g *a.i.*/ha as pre-emergence, diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS, imazethapyr 10% SL @ 100 g *a.i.*/ha (PoE) at 15 -20 DAS, sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix)@ 165 +80 g *a.i.*/ha at 20 DAS, propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g *a.i.*/ha at 20-25DAS (RM), two hand

weeding at 20 & 40 DAS and weedy check was laid out in randomized block design with three replications.

RESULTS AND DISCUSSION

1. Efficacy of herbicides on growth of soybean under weed control treatments

Plant height & branches/plant

In general, the plant height enlarged with the development in crop growth stages, irrespective of the treatment and reached maximum at harvest. The data shown in Table 1.1 indicated that there was no significant variation in plant height across different treatments at 30 days after sowing (DAS).

Plant height increase quickly between 30-60 days in all the treatment varying from 29.39 to 50.11 cm at 60 DAS. Significantly taller plants were observed under two hand weeding at 20 and 40 DAS (50.11 cm), followed by application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS (45.71cm), it was at par with pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (45.05 cm), over weedy check. Respective treatments recorded 37.70, 25.61 and 23.79 per cent more plant height over weedy check.

Two hand weeding constantly recorded the taller plants at harvest (64.57 cm) as compared to all other treatments. The trend was nearly parallel as recorded at 60 DAS. The plant height under different treatments varied from 44.54 to 64.57 cm. Hand weeding twice, diclosulam 26 g *a.i./ha* PE+ 1 HW,

pyroxasulfone 127.5 g *a.i./ha* PE + 1 HW treatment recorded 44.97, 39.26 and 34.97 per cent more plant height over weedy check.

Based on the data from Table 1.1 the number of branches/plant at harvest was significantly higher under two hand weeding treatments (3.60) compared to all herbicidal treatments and the weedy check.

Among the herbicidal treatments, the application of diclosulam at 26 g *a.i./ha* pre-emergence, combined with one hand weeding at 40 DAS, significantly recorded a higher number of branches/plant (3.13). This was at par with the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence, also combined with one hand weeding at 40 DAS, which resulted in 2.93 branches/plant, and superior to the rest of the herbicidal treatments. Respective treatments were recorded 62.17 and 51.83 per cent more branches/plant over weedy check.

Additionally, the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence, combined with one hand weeding at 40 DAS, found statically at par in respect to numbers of branches/plant (2.93) with application of sodium acifluorfen 16.5% + clodinafop propargyl 8% premix at 165 + 80 g *a.i./ha* at 20 DAS (2.87), propaquizafop 2.5% + imazethapyr 3.75% ME at 50 + 75 g *a.i./ha* at 20–25 DAS (2.80) and the application of imazethapyr 10% SL at 100 g *a.i./ha* post-emergence at 15–20 DAS (2.73). These herbicidal treatments were superior to the weedy check, which

was recorded the minimum number of branches/plant at harvest (1.93).

The aspects of plant height and branch/plant increased with an increase in the age of the plant up to 75 DAS and thereafter it remained almost stagnant till the harvest time, it was evident that all weed control measure enhanced significantly plant height and branches/plant compared to weedy check. The treatment where hand weeding was done twice, at 20 and 40 DAS recorded the highest plant height and the highest number of branches/plant, followed by the application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS, which was at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence + one hand weeding at 40 DAS treatment combined with one hand weeding at 40 DAS. The increase in the values of both parameters in response to the herbicidal weed control treatments might be as a result of less competition from the crop due to reduction in the number of weeds as a result of suppression of root and shoot growth of the weeds. Thus, competition by crops with weeds for light, nutrients, moisture and space was minimized and the clear effect was that each crop plant had a better growing environment in the absence of weeds. These results support the findings of Gupta and Chandrakar (2014), Jitendra *et al.* (2022).

Root nodules

Data showed in Table 1.1 at 50 DAS, the number of root nodules/plant recorded significantly higher under two hand

weeding (53.0) over weedy check and followed by application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding recorded significantly higher number of root nodules/plant 40 DAS (48.74), which was at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence + one hand weeding at 40 DAS (45.41) and superior over rest of the chemical weed control treatments. Application of pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence + one hand weeding at 40 DAS (45.41) was on par with sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix)@ 165 +80 g *a.i.*/ha at 20 DAS (44.39), propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g *a.i.*/ha at 20 – 25 DAS (RM) (43.86) and application of imazethapyr 10% SL @ 100 g *a.i.*/ha (PoE) at 15 -20 DAS (43.80), superior over rest of the herbicidal treatments and weedy check. Two hand weeding, diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS and pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence + one hand weeding at 40 DAS was recorded 64.54, 51.31 and 40.98 per cent more root nodules/plant over weedy check. Lowest number of root nodules/plant was recorded under weedy check (32.21).

This parameter was recorded higher under the treatment involving hand weeding twice at 20 and 40 DAS, followed by the application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS, which was at par with application

of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS. This variability among the different treatments can be attributed to the competition between crops and weeds for space, nutrients, and light. Decreased competition due to fewer weeds provides more rhizospheric space for the crop, leading to more effective nodulation in both quantity and quality. These results are consistent with the findings of Kalyani *et al.* (2023).

Dry matter accumulation by crop

The data indicated in Table 1.1 that significantly higher dry matter accumulation at 30 DAS by crop in g/plant was observed under two hand weeding (2.23 g). Among herbicidal treatments, application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS significantly recorded higher dry matter accumulation g/plant (2.08) being at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (1.92 g), superior over weedy check and rest of the herbicidal treatments. Application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (1.92 g) was on par with sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix)@ 165 +80 g *a.i./ha* at 20 DAS (1.82 g), propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g *a.i./ha* at 20 – 25 DAS (RM) (1.80) and imazethapyr 10% SL @ 100 g *a.i./ha* (PoE) at 15 -20 DAS (1.75 g). The lowest value was recorded under weedy check (0.88g).

At 60 DAS, twice hand weeding was recorded highest dry matter accumulation (13.95 g) by crop in g/plant, which was significantly higher as compared to all herbicidal treatments and weedy check (4.03 g). Among herbicidal treatments, application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS recorded significantly higher dry matter accumulation g/plant (12.02 g) being at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (11.39 g) and superior over control and rest of the chemical weed control treatments.

The dry matter accumulation by crop was recorded significantly higher under two hand weeding (25.84 g) at harvest, which was superior over all weed control treatments. Among herbicidal treatments, application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS recorded significantly higher dry matter accumulation g/plant (22.94 g), over weedy check and rest of the herbicidal treatments, except application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (21.12 g). Lowest dry matter accumulation by crop was recorded under weedy check (10.19 g). Hand weeding twice, diclosulam 26 g *a.i./ha* PE+ 1 HW, pyroxasulfone 127.5 g *a.i./ha* PE + 1 HW treatment recorded 153.58, 125.12 and 107.26 per cent more dry matter accumulation at harvest over weedy check.

Minimum dry matter accumulation (g/plant) by the crop was observed in the weedy check at 30, 60 DAS, and at harvest, primarily due to intense competition between the crop and weeds for essential growth resources during the critical growth period. However, significant increases were noted when weed control measures were implemented in various plots. Specifically, plots that received hand weeding twice at 20 and 40 DAS exhibited notably enhanced dry matter accumulation by the crop, followed by the application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS, which was at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS treatment combined with one hand weeding at 40 DAS, and the application of sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix) at 165 + 80 g *a.i./ha* at 20 DAS. "This increase is credited to decreased weed competition achieved through effective weed control, facilitating improved growth and development of plant parts. Consequently, this resulted in higher dry matter accumulation by the crop compared to other herbicidal treatments. Comparable results were documented by Jitendra *et al.* (2022) and Patil *et al.* (2023). Who also found higher dry matter accumulation in plots treated with two hand weeding followed by pre-emergence application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS.

2. Yield attributes and yield

Pods/plant

Based on the data presented in Table 1.2 all weed control treatments significantly increased the number of pods/plant compared to the weedy check (23.27 pods/plant). Hand weeding twice resulted in the highest number of pods/plant at (48.93).

Among the herbicidal treatments, the application of diclosulam at 26 g *a.i./ha* pre-emergence combined with one hand weeding at 40 days after sowing (DAS), achieved a significantly higher number of pods/plant at (45.20). This was comparable to the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence, also combined with one hand weeding at 40 DAS, which recorded (43.43) pods/plant and outperformed other weed control treatments. Respective treatments were recorded 94.24 and 86.63 per cent more pods/plant over weedy check.

Furthermore, the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence combined with one hand weeding at 40 DAS, showed almost similar numbers of pods/ plant (43.43) as sodium acifluorfen 16.5% + clodinafop propargyl 8% premix at 165 + 80 g *a.i./ha* at 20 DAS (41.53), propaquizafop 2.5% + imazethapyr 3.75% ME at 50 + 75 g *a.i./ha* at 20–25 DAS (39.67) and the application of imazethapyr 10% SL at 100 g *a.i./ha* post-emergence at 15–20 DAS (39.07). In other herbicidal treatments, the number of pods/plant ranged from 23.26 to 37.96.

Competition between crops and weeds impacts crucial physiological parameters, leading to decreased yield attributes and overall crop yield. Early season weed control and maintaining a weed-free environment mitigate crop-weed competition stress, significantly enhancing yield. The strong correlation observed between seed yield and the number of pods/plant ($r = 0.927^{**}$) also validates profound effects of these parameters on yield. Hand weeding twice at 20 and 40 DAS resulted in higher pods/plant compared to other treatments, followed by the application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS, which was statically at par with the application of pyroxasulfone 85% WG at 127.5 g *a.i.*/ha as a pre-emergence treatment combined with one hand weeding at 40 DAS. The lowest number of pods/plant was observed in the weedy check, a finding consistent with results reported by Kalyani *et al.* (2023) and Borje *et al.* (2023).

Seeds/pod

The findings in Table 1.2 that the number of seeds/pods was found significant among all the different treatments. Two hand weeding recorded maximum number of seeds/pod (2.73) followed by application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS (2.53). The number of seeds/pod varied between 1.73 to 2.73 under rest of the treatments.

Reveals that the various weed control treatments had no significant effects on seed index. The highest values

for seed/pod were observed in plots treated with hand weeding twice at 20 and 40 DAS, which were comparable to the other treatments. Conversely, the lowest values for seeds/pod were noted in the weedy check. These findings align with those reported by Kalyani *et al.* (2023).

3. Efficacy of herbicides on yields of soybean under weed control treatments

Seed yield

The data presented in Table 1.2 demonstrated that all weed control treatments resulted in significantly higher seed yields compared to the weedy check (732 kg/ha). Twice hand weeding recorded the maximum seed yield (2005 kg/ha), surpassing all herbicidal treatments.

Among the herbicidal treatments, the application of diclosulam at 26 g *a.i.*/ha pre-emergence combined with one hand weeding at 40 DAS, achieved a significantly higher seed yield of 1783 kg/ha, comparable to the application of pyroxasulfone 85% WG at 127.5 g *a.i.*/ha pre-emergence also combined with one hand weeding at 40 DAS, which yielded (1686 kg/ha) and outperformed all other herbicidal treatments. Two hand weeding at 20 & 40 DAS, diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS and pyroxasulfone 85 % WG @ 127.5 g *a.i.*/ha as pre-emergence + one hand weeding at 40 DAS was recorded 173.90, 143.57 and 130.32 more per cent seed yield, respectively, over weedy check.

Additionally, the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence combined with one hand weeding at 40 DAS, showed comparable seed yields (1686 kg/ha) to sodium acifluorfen 16.5% + clodinafop propargyl 8% premix at 165 + 80 g *a.i./ha* at 20 DAS (1533 kg/ha), propaquizafop 2.5% + imazethapyr 3.75% ME at 50 + 75 g *a.i./ha* at 20–25 DAS (1500 kg/ha) and application of imazethapyr 10% SL at 100 g *a.i./ha* post-emergence at 15–20 DAS (1492 kg/ha), respectively,

The lowest seed yield among herbicidal treatments was recorded under the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence (1120 kg/ha).

Yield is a complex phenomenon influenced by the genetic potential of the crop and the management practices implemented during growth and development. Weeds can cause substantial damage to crops depending on the weed species present, their density, the duration of competition with the crop, and their cumulative effect, all of which contribute to reduced crop yield. In soybeans, yield attributes such as the number of pods/plant, number of seeds/plant, and seed index are crucial for determining yield potential. Crop-weed competition has been observed to decrease these yield attributes in soybeans, resulting in significant reductions in seed yield, as reported by Godec and Opacic (1998). The current study similarly demonstrated a significant reduction in all yield components in the weedy check. Thus,

crop-weed competition adversely affects important physiological parameters, leading to reductions in various yield components.

The data from Table 1.2 indicate that all weed control treatments significantly increased seed yield compared to the weedy check. Hand weeding twice at 20 and 40 DAS resulted in the highest seed yield among all treatments. This method promoted robust crop growth by removing weeds from both inter and intra rows, improving soil aeration, and thereby optimizing space, water, light, and nutrient availability for enhanced growth and development. These favorable conditions contributed to superior yield attributes and ultimately the highest overall yield. Several researchers, including Kalyani *et al.* (2023), Meena *et al.* (2022), Meena *et al.* (2012), and Ahirwar and Ahirwar (2023) have reported similar findings, underscoring the effectiveness of hand weeding in achieving maximum soybean yield.

Among herbicidal treatments, application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS recorded significantly higher seed yield, which was statistically at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS. Apon and Nongmaithem (2022) and Kotnake and Goud (2023) also reported diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS gave higher yield.

Straw yield

It is evidenced from the data presented in Table 1.2 discovered that all the weed control treatments caused significantly superior straw yield as compared to weedy check (1091 kg/ha). Two hand weeding recorded highest straw yield (2854 kg/ha), which was superior over all herbicidal treatments. Among herbicidal treatments, application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS was recorded significantly higher straw yield (2558 kg/ha) being at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (2428 kg/ha) and superior over rest of the treatments. Application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (2428 kg/ha) was on par with sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix)@ 165 +80 g *a.i./ha* at 20 DAS (2218 kg/ha), propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g *a.i./ha* at 20 – 25 DAS (RM) (2180 kg/ha) and application of imazethapyr 10% SL @ 100 g *a.i./ha* (PoE) at 15 -20 DAS (2171 kg/ha). Two hand weeding at 20 & 40 DAS, diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS and pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS was recorded 161.59, 134.46 and 122.54 more straw yield, respectively, over weedy check.

Biological yield

The data from Table 1.2 indicate that all weed control treatments resulted in significantly higher biological yields compared to the weedy check (1823 kg/ha). The highest biological yield (4859 kg/ha) was achieved with two hand weeding, which surpassed all herbicidal treatments.

Among the herbicidal treatments, the application of diclosulam at 26 g *a.i./ha* pre-emergence combined with one hand weeding at 40 DAS showed a significantly higher biological yield (4341 kg/ha). This was comparable to the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence, also combined with one hand weeding at 40 DAS (4114 kg/ha) and outperformed over other chemical weed control treatments. Two hand weeding at 20 & 40 DAS, diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS and pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS treatments were recorded 166.48, 138.12 and 125.67 more biological yield over weedy check respectively.

Additionally, in reference to biological yield, the application of pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence + one hand weeding at 40 DAS (4114 kg/ha) was found at par with sodium acifluorfen 16.5% + clodinafop propargyl 8% premix at 165 + 80 g *a.i./ha* at 20 DAS (3751 kg/ha), propaquizafop 2.5% + imazethapyr 3.75% ME at 50 + 75 g *a.i./ha* at 20–25 DAS (3680 kg/ha), and

the application of imazethapyr 10% SL at 100 g *a.i./ha* post-emergence at 15–20 DAS (3663 kg/ha). Biological yields in other herbicidal treatments ranged from (2773 to 2910 kg/ha).

The increase in straw yield is directly correlated with the vegetative growth of the plant. The various weed control treatments had a significant effect on the straw yield and biological yield of soybean. Hand weeding twice at 20 and 40 DAS resulted in the highest straw and biological yield. Among the herbicidal treatments, the application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS recorded the highest straw yield, which was statistically at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS. The results obtained in the present investigation are in close agreement with the findings of Kalyani *et. al.*(2023), Aher *et.al.*(2023) and Ahirwar and Ahirwar (2023).

4. Efficacy of herbicides on quality of soybean under weed control treatments

Oil content and oil yield

The data showed in Table 1.3 revealed that during course of investigation, different weed control treatments failed to bring any significant variation in oil content in soybean seed. The maximum oil content (%) and oil yield was recorded in two hand weeding (20.57 % and 412.19 kg/ha) followed by application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS (20.19 % and oil yield 359.85

kg/ha), respectively. The minimum oil content (19.28%) and oil yield (141.53 kg/ha) was recorded under weedy check. The oil content and oil yield in other herbicidal treatments varied between (19.65 to 20.1%) and (220.27 to 337.32 kg/ha). Two hand weeding at 20 & 40 DAS, diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS and pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS treatments were recorded 191.23, 154.25 and 138.23 per cent more oil yield over weedy check, respectively.

A review of the data from Table 1.3 revealed that all weed control treatments did not significantly impact the oil content of soybean seeds. However, oil yield significantly increased due to weed control treatments. Oil yield is mostly a function of seed yield. Robust positive correlation between oil yield and seed yield ($r = 0.999^{**}$) support the fact. Hand weeding twice at 20 and 40 DAS resulted in the highest oil yield, followed by the application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS, which was statistically similar to the application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS. Application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS was found at par with application of sodium acifluorfen 16.5% + clodinafop propargyl 8% (premix) at 165 + 80 g *a.i./ha* at 20 DAS, and the application of propaquizafop 2.5% + imazethapyr

3.75% ME at 50 + 75 g *a.i./ha* at 20–25 DAS (RM), all of which were significantly superior to the weedy check. These results are consistent with the findings of Jadon *et al.* (2019) and Pawar *et al.* (2022).

Protein content and protein yield

Based on the data presented in Table 1.3, all weed control treatments showed significantly higher protein content in seeds compared to the weedy check (36.60%). Specifically, two hand weeding treatments recorded the highest protein content in seeds (42.10%). Additionally, the application of diclosulam at 26 g *a.i./ha* pre-emergence combined with one hand weeding at 40 DAS, recorded significantly higher protein content of 41.29 per cent compared to the weedy check and other chemical weed control treatments, except with pyroxasulfone 85% WG at 127.5 g *a.i./ha* pre-emergence + one hand weeding at 40 DAS, which recorded (40.69%) protein content. The lowest protein content was observed under the weedy check condition (36.60%).

Significantly higher protein yield was produced under two hand weeding (843.52 kg/ha) as compared to all weed control treatments. Among herbicidal weed control treatments, application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS recorded significantly higher protein yield (736.52 kg/ha) over weedy check and rest of the herbicidal treatments, except application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS (685.98 kg/ha). Two hand weeding at 20 & 40 DAS, diclosulam @ 26 g *a.i./ha* at pre-

emergence + one hand weeding at 40 DAS and pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS was recorded 214.78, 174.85 and 155.99 per cent higher protein yield, respectively, over weedy check.

The data from Table 1.3 demonstrate that all weed control treatments had a significant impact on the protein content of soybean seeds. Protein yield notably increased as a result of these weed management practices, given that protein yield is largely influenced by seed yield. Strong positive correlation coefficient value between protein yield and seed yield ($r = 0.998^{**}$) support the fact. Hand weeding twice at 20 and 40 DAS recorded the highest protein content, followed by application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS. Data further revealed that two hand weeding at 20 and 40 DAS registered highest protein yield followed by application of diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS, which was statistically at par with application of pyroxasulfone 85 % WG @ 127.5 g *a.i./ha* as pre-emergence + one hand weeding at 40 DAS and significantly superior over weedy check. Jadon *et al.* (2019) and Pawar *et al.* (2022) was also reported highest protein yield under two hand weeding.

CONCLUSION

The results of one-year experiment revealed that weed control by two hand weeding at 20 & 40 DAS produced the higher growth, yield and quality parameter of soybean and followed by diclosulam @ 26 g *a.i./ha* at pre-emergence + one hand weeding at 40 DAS, however higher growth, yield and quality parameter were

obtained by application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS.

Based on yield and economics consideration, it can be concluded that

weed in soybean may be controlled by pre-emergence application of diclosulam @ 26 g *a.i.*/ha at pre-emergence + one hand weeding at 40 DAS

Table 1.1 Effect of herbicides on plant height, branches/plant, Root nodules/plant and dry matter accumulation at different growth stages

Treatment	Plant height (cm)			branches /plant	Root nodules/plant	Dry matter accumulation (g/plant)		
	30 DAS	60 DAS	At harvest	At harvest	At 50 DAS	30 DAS	60 DAS	At harvest
T1. Pendimethalin 30% EC + Imazethapyr 2% SL @ 1.0 kg a.i./ha (PE) (RM)	21.37	36.01	46.62	2.67	37.43	1.5	8.32	17.46
T2. Pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence	21.17	35.71	46.19	2.47	36.18	1.33	7.17	15.73
T3. Pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence + one hand weeding at 40 DAS	22.87	45.05	60.12	2.93	45.41	1.92	11.39	21.12
T4. Diclosulam @ 26 g a.i./ha as pre-emergence	21.31	35.85	46.4	2.6	36.71	1.4	7.78	16.45
T5. Diclosulam @ 26 g a.i./ha at pre-emergence + one hand weeding at 40 DAS	23.13	45.71	62.03	3.13	48.74	2.08	12.02	22.94
T6. Imazethapyr 10% SL @ 100 g a.i./ha (PoE) at 15 -20 DAS	21.5	40.41	53.35	2.73	43.8	1.75	10.46	19.52
Continous table 1								
T7. Sodium acifluorfen 16.5% + Clodinafop propargyl 8% (premix)@ 165 +80 g a.i./ha at 20 DAS	22.67	44.82	54.74	2.87	44.39	1.82	10.75	20.58
T8. Propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g a.i./ha at 20 – 25 DAS (RM)	22.65	44.53	54.33	2.8	43.86	1.8	10.59	19.76
T9. Two hand weeding at 20 & 40 DAS	23.89	50.11	64.57	3.6	53	2.23	13.95	25.84
T10. Weedy check	20.89	36.39	44.54	1.93	32.21	0.88	4.53	10.19
SEm±	1.04	1.71	2.19	0.15	1.63	0.07	0.39	0.7
CD (P=0.05)	NS	5.09	6.51	0.45	4.83	0.21	1.16	2.09
CV %	8.13	7.16	7.13	9.52	6.67	7.2	6.99	6.44

Table 1.2 Effect of herbicides on yield attributes and yields of soybean

Treatment	Number of pod/plant	Number of seed /pod	Seed yield (kg/ha)	Straw yield (kg/ha)	Biological yield (kg/ha)
T ₁ . Pendimethalin 30% EC + Imazethapyr 2% SL @ 1.0 kg a.i./ha (PE) (RM)	38.77	2.22	1183	1727	2911
T ₂ . Pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence	37.87	2.13	1120	1653	2773
T ₃ . Pyroxasulfone 85 % WG @ 127.5 g a.i./ha as pre-emergence + one hand weeding at 40 DAS	43.43	2.47	1686	2428	4114
T ₄ . Diclosulam @ 26 g a.i./ha as pre-emergence	37.93	2.2	1157	1706	2862
T ₅ . Diclosulam @ 26 g a.i./ha at pre-emergence + one hand weeding at 40 DAS	45.2	2.53	1783	2558	4341
T ₆ . Imazethapyr 10% SL @ 100 g a.i./ha (PoE) at 15 -20 DAS	39.07	2.27	1492	2171	3663
T ₇ . Sodium acifluorfen 16.5% + Clodinafop propargyl 8% (premix)@ 165 +80 g a.i./ha at 20 DAS	41.53	2.4	1533	2218	3751
T ₈ . Propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g a.i./ha at 20 – 25 DAS (RM)	39.67	2.33	1500	2180	3680
T ₉ . Two hand weeding at 20 & 40 DAS	48.93	2.73	2005	2854	4858
T ₁₀ . Weedy check	23.27	1.73	732	1091	1823
SEm±	1.83	0.11	74.06	115.5	187.31
CD (P=0.05)	5.43	0.33	220.05	343.16	556.54
CV %	8	8.44	9.04	9.72	9.33

Table 1.3 Effect of herbicides on oil and protein content and their yield in soybean

Treatment	Oil content (%)	Oil Yield (kg/ha)	Protein content (%)	Protein Yield (kg/ha)
T ₁ . Pendimethalin 30% EC + Imazethapyr 2% SL @ 1.0 kg a.i/ha (PE) (RM)	19.65	220.27	39.04	461.85
T ₂ . Pyroxasulfone 85 % WG @ 127.5 g a.i/ha as pre-emergence	19.68	227.74	38.54	431.46
T ₃ . Pyroxasulfone 85 % WG @ 127.5 g a.i/ha as pre-emergence + one hand weeding at 40 DAS	20.01	337.32	40.69	685.98
T ₄ . Diclosulam @ 26 g a.i/ha as pre-emergence	19.75	233.24	38.63	446.84
T ₅ . Diclosulam @ 26 g a.i/ha at pre-emergence + one hand weeding at 40 DAS	20.19	359.85	41.29	736.52
T ₆ . Imazethapyr 10% SL @ 100 g a.i /ha (PoE) at 15 -20 DAS	19.8	295.54	39.35	587
T ₇ . Sodium acifluorfen 16.5% + Clodinafop propargyl 8% (premix)@ 165 +80 g a.i/ha at 20 DAS	19.93	305.83	40.42	618.79
T ₈ . Propaquizafop 2.5% + imazethapyr 3.75 %ME @50 + 75g a.i/ha at 20 – 25 DAS (RM)	19.82	297.39	39.6	593.67
T ₉ . Two hand weeding at 20 & 40 DAS	20.57	412.19	42.1	843.52
T ₁₀ . Weedy check	19.28	141.53	36.6	267.97
Sem±	0.36	15.35	0.75	30.47
CD (P=0.5)	NS	45.6	2.23	90.52
CV %	3.13	9.39	3.28	9.3

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Identification of Polymorphic Markers for Combining Photoperiod Insensitivity and Long Juvenility in Soybean Breeding

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ABSTRACT

Marker-assisted backcrossing requires the identification of polymorphic markers between recipient and donor parents to facilitate background and recombinant selection. This study is a part of a research work undertaken to transfer the long juvenile e9 allele from the germplasm line LJ 140 into photoperiod-insensitive soybean varieties. These varieties include e1-del (NRC 138), e2 (JS 20-34), and e3 (NRC 130), as well as the germplasm line NRC 204 carrying the e1-as allele. A total of 638 simple sequence repeat (SSR) markers distributed across 20 linkage groups were screened for polymorphism across four parental combinations involving donor LJ 140 and recipients NRC 204, JS 20-34, NRC 130, and NRC 138. The mean polymorphism rates observed were 24.7% for NRC 130 × LJ 140, 24.7% for NRC 138 × LJ 140, 26.0% for NRC 204 × LJ 140, and 22.4% for JS 20-34 × LJ 140. These polymorphic markers will enable efficient background selection, ensuring higher recovery of the recipient genome and expediting the development of soybean varieties that combine photoperiod insensitivity with long juvenility.

Keyword: Marker-assisted breeding (MAS), SSR Marker, Polymorphism, Photoperiod Insensitivity, Long Juvenility

Soybean (*Glycine max*) is among the most important seed legumes globally; contributing approximately 25% of the world's edible oil supply and 65% of the protein in animal feed concentrates(Kumar et al., 2021). In India, soybean has rapidly established itself as a significant oilseed crop, cultivated on about 10 million hectares(Nair et al., 2023). The crop is

particularly valued for its adaptability to different agro-climatic conditions and its role in sustaining the agricultural economy. A unique feature of soybean is its sensitivity to photoperiod, with flowering being triggered when the day length falls below a genotype-specific critical day length (CDL) (Hadley et al., 1984). This sensitivity is determined by the plant's genetic makeup and varies

across latitudes and seasons, allowing soybean to grow in a wide range of environments, from 50°N to 45°S (Watanabe et al., 2012). The flowering and maturity traits are controlled by 12 major loci, designated as E1 to E11 and J, which influence the plant's response to photoperiod and developmental timing (Samanfar et al., 2017). Genes such as E1, E2, E3, and E4 are photoperiod-responsive, delaying flowering under long-day conditions, while E9 and J, which are associated with the long juvenile trait, delay flowering under short-day conditions to extend vegetative growth (Upadhyay et al., 1994a, 1994b). This intricate genetic control enables soybean to adapt to a wide range of environmental conditions but also poses challenges for breeding varieties suited to specific climates.

In India's Central Zone, which accounts for 93% of the country's soybean production, the crop is primarily grown as a short-duration rainfed crop, sown with the onset of the monsoon in June. However, erratic rainfall and delayed monsoons, sometimes stretching into July, lead to shorter day lengths during the early stages of growth. This often results in premature flowering in photoperiod-sensitive varieties, reducing plant height and yield potential (Ort et al., 2022). To counter these challenges, varieties with long juvenile alleles, which delay flowering under short-day conditions, have been identified. These varieties enable greater biomass accumulation and yield, but their delayed maturity makes them less attractive for farmers seeking early harvests. Conversely, photo-insensitive

varieties, which flower and mature early, often exhibit reduced yield and biomass under short-day conditions. This trade-off highlights the need for innovative breeding programs to develop soybean genotypes that combine photo-insensitivity with long juvenile traits. Such varieties would provide greater adaptability to varying sowing dates, environmental conditions, and farmer requirements. Moreover, this approach would stabilize yields and improve resilience to climate-induced challenges, ensuring that soybean remains a reliable source of edible oil and animal feed. As the demand for soybean continues to rise globally, particularly in tropical regions like India, these genetic advancements are crucial for supporting sustainable agricultural growth.

Soybean varieties with photoperiod insensitivity, such as NRC 138 (e1-del), JS 20-34 (e2), and NRC 130 (e3), have been successfully developed in India. However, varieties incorporating the long juvenile trait are not yet available. This research aims to introgress the e9 long juvenile allele from the LJ 140 germplasm line into these photoperiod-insensitive varieties through molecular marker-assisted breeding. Marker-assisted backcrossing relies heavily on the availability of polymorphic markers that provide comprehensive genome coverage. Among the various marker systems, Simple Sequence Repeats (SSRs) remain the preferred choice due to their broad genomic distribution, high genome coverage, cost-efficiency, co-dominant inheritance, strong reproducibility, high polymorphism, and suitability for marker-assisted

selection (MAS). This study identifies polymorphic SSR markers across parental combinations involving the long juvenile donor LJ 140 and recipients NRC 130, JS 20-34, NRC 138, and NRC 204.

MATERIALS AND METHODS

The present study was conducted at the ICAR-Indian Institute of Soybean Research (ICAR-IISR), Indore, Madhya Pradesh, India. Seeds of the recipient genotypes NRC 130, NRC 138, and JS 20-34 were procured from the All India Coordinated Research Project on Soybean, based at ICAR-IISR Indore. Seeds of LJ 140 and NRC 204 were obtained from the germplasm section of ICAR-IISR Indore. LJ 140, a line developed at ICAR-IISR, carries the null allele for long juvenility (e9) and a hypoactive allele for photoperiod insensitivity (e1-as). The recipient genotypes—NRC 130, NRC 138, and JS 20-34—are early-maturing soybean varieties adapted to the Central Zone of India, while NRC 204 is a near-isogenic line of the SL 958 (E1) variety, harboring the e1-as allele.

DNA Isolation

Genomic DNA was extracted from fresh tender leaves of each genotype using the cetyltrimethylammonium bromide (CTAB) method using the protocol of (Doyle, JJ; Doyle, 1990). The sample was taken in eppendorf tube then crushed by using Tissue Lyser for 5 minutes with 900µl of CTAB extraction buffer. Eppendorf tubes containing the samples were incubated at 60°C for 1 hour in a water bath with

occasional shaking. After incubation, the samples were brought to room temperature, and 800 µl of chloroform: isoamyl alcohol (24:1) was added. The samples were then thoroughly mixed for approximately 10 minutes to ensure homogeneity and subsequently centrifuged at 10,000 rpm for 10 minutes at room temperature.

The upper layer from the centrifuged samples was carefully collected into fresh tubes, and $\frac{3}{4}$ volume of isopropanol was added to the collected layer and gently mixed. The tubes were incubated at 4°C for 2 hours, followed by centrifugation at 10,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the resulting pellet was washed twice with 70% ethanol by centrifuging at 10,000 rpm for 10 minutes at 4°C. Finally, the pellet was air-dried at 37°C for 1 hour and dissolved in TE buffer for further use.

SSR Marker based PCR

For the PPS study, a total of 638 SSR marker pairs spanning all 20 soybean linkage groups were analyzed. This approach ensures comprehensive coverage of all chromosomes and maintains a consistent distribution of markers across the genome.

The DNA was subjected to polymerase chain reaction (PCR) amplification in 96 well plate comprising 1.5 µL of DNA, 1 µL of 10× PCR buffer, 0.5 µL of MgCl₂ (25 mM), 0.25 µL of deoxynucleotide triphosphates (dNTPs) (25 mM), 0.5 µL each of forward and reverse SSR primers, 0.1 µL of Taq DNA polymerase (5 U/µL), and 5.65

μL of PCR-grade double-distilled water to make the total volume of 10μl for each reaction.

The amplification protocol included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 60 seconds, and elongation at 72°C for 60 seconds. A final elongation step was performed at 72°C for 5 minutes.

Agarose gel electrophoresis and gel documentation

DNA purity was assessed by visualizing it on a 0.8% agarose gel. PCR products were resolved on a 3.5% agarose gel stained with the nucleic acid stain Goodview and visualized using a gel documentation system.

RESULT AND DISCUSSION

The parental polymorphism between the four parental combinations was determined by analyzing the differences in base pair sizes for both parents. The percentage of polymorphism was calculated using the following formula-

$$\text{Polymorphism \%} = \frac{\text{Number of polymorphic marker identified}}{\text{Total number of marker run}} \times 100$$

A total of 638 SSR markers spanning the genome and covering all 20 chromosomes were analyzed to assess polymorphism among the parental combinations. A subset of these markers is presented for linkage groups A2 and B2 (Figure 1). Among these, 158, 158, 166, and 143 SSR markers were found to be polymorphic in the parental combinations NRC 130 × LJ

140, NRC 138 × LJ 140, NRC 204 × LJ 140, and JS 20-34 × LJ 140, respectively (Table 1). This corresponds to polymorphism rates of 24.76%, 24.76%, 26.01%, and 22.41%, respectively. These results highlight the genetic variability between the parents in each combination, which will aid in efficient marker-assisted recovery of the recurrent genome in backcrossed generations.

Furthermore, 36 SSR markers distributed across different linkage groups were found to be polymorphic across all four parental combinations (Table 2). Among these, the highest number of common polymorphic markers was observed in linkage group D1a(chr1), indicating a non-uniform distribution of polymorphic markers across the genome.

The distribution of polymorphic markers within each parental combination is shown in Figure 2 for NRC 130 × LJ 140, NRC 138 × LJ 140, NRC 204 × LJ 140, and JS 20-34 × LJ 140. The highest percentage of polymorphism 43.48% for NRC 130 × LJ 140 was found on linkage group M(chr7) with polymorphism ranged from 5.56% to 43.48%(Table 3). For NRC 138 × LJ 140, highest percentage of polymorphism 41.67% was found on linkage group D1a (chr1) with polymorphism ranging from 5.56% to 41.67%(Table3). For NRC 204 × LJ 140, highest polymorphism was found on linkage group M(chr7) with polymorphism ranging from 5.56% to 43.48% (Table3) and for JS 20-34 × LJ 140 highest percentage of polymorphism 45.83% was found on

linkage group D1a(chr1) with polymorphism ranging from 2.78% to 45.83%(Table3). This analysis underscores the variation in marker distribution across the genome and provides valuable resources for genotyping and mapping studies in soybean breeding programs.

Dinucleotide SSR repeats motifs were observed to be more polymorphic than trinucleotide and tetranucleotide repeats across all parental combinations (Figure 3). The total counts of dinucleotide repeats identified were 117, 119, 127, and 101 in NRC 130 × LJ 140, NRC 138 × LJ 140, NRC 204 × LJ 140, and JS 20-34 × LJ 140, respectively. In comparison, trinucleotide repeats numbered 40, 37, 38, and 40, while tetranucleotide repeats were notably sparse, with only 1, 2, 2, and 3, respectively, across the same combinations. These findings align with observations by (Narshimulu et al., 2011) who reported that dinucleotide repeats were the most prevalent in rice, followed by trinucleotide and tetranucleotide repeats. The predominance of dinucleotide repeats suggests they are either evolutionarily favored or more prone to

polymorphism. In contrast, trinucleotide repeats are less common, and tetranucleotide repeats are rare, supporting the hypothesis that smaller repeat units, such as dinucleotides, are both more abundant and polymorphic.

Polymorphic SSR markers are widely used in marker-assisted breeding (MAS). Kim et al., 2008 developed five near-isogenic lines (NILs) for the rxp trait using MAS. In 2020, the SNP marker KSS-SNP5 on chromosome 16 was identified as crucial for pod shattering resistance and integrated into soybean breeding programs (Kim et al., 2020). Additionally, MAS identified promising soybean genotypes (e1 e3 E4 e7, e1 E3 E4 e7, and e1 E3 e4 e7), showing earlier maturity at 53°N in northern Kazakhstan, as reported by (Yerzhebayeva et al., 2023).

The polymorphic SSR markers identified in this study can serve as valuable tools for genotyping backcrossed progeny in breeding programs. By selecting SSR markers based on their polymorphism and repeat motif distribution, the precision and effectiveness of genetic analysis can be significantly enhanced.

Table 1: PPS of parental combination using 638 SSR markers

S.no	Parental combination	Total Number of Markers	Number of total polymorphic marker	Total percentage polymorphism
1	NRC130 × LJ 140	638	158	24.76%
2	NRC 138 × LJ 140	638	158	24.76%
3	NRC204 × LJ 140	638	166	26.01%
4	JS20-34 × LJ 140	638	143	22.41%

Table 2: Details of common polymorphic SSR markers for all four parental combinations across different Linkage groups.

Linkage Group	Number of polymorphic SSR marker	SSR Marker
A1	4	BARCSOYSSR_05_0799, BARCSOYSSR_05_1014, BARCSOYSSR_05_1016, BARCSOYSSR_05_1020
A2	1	BARCSOYSSR_08_1730
B1	0	
B2	2	BARCSOYSSR_14_1444, BARCSOYSSR_14_1531
C1	1	BARCSOYSSR_04_1516
C2	4	BARCSOYSSR_06_0980, BARCSOYSSR_06_0986, BARCSOYSSR_06_1086, BARCSOYSSR_06_1155
D1a	5	BARCSOYSSR_01_0117, BARCSOYSSR_01_0449, BARCSOYSSR_01_1409, BARCSOYSSR_01_1561, BARCSOYSSR_01_1782
D1b	3	BARCSOYSSR_02_0482, BSSRBARCSOYSSR_02_793, BARCSOYSSR_02_0958
D2	0	
E	4	BARCSOYSSR_15_0157, BARCSOYSSR_15_0582, BARCSOYSSR_15_0801, BARCSOYSSR_15_1455
F	2	BARCSOYSSR_13_0358, BARCSOYSSR_13_0792
G	2	BARCSOYSSR_18_1536, BARCSOYSSR_18_1542
H	1	BARCSOYSSR_12_1092
I	0	
J	2	BARCSOYSSR_16_1010, BARCSOYSSR_16_1064
K	0	
L	2	BARCSOYSSR_19_1252, BARCSOYSSR_19_1466
M	3	BARCSOYSSR_07_0386, BARCSOYSSR_07_0502, BARCSOYSSR_07_0718
N	0	
O	0	

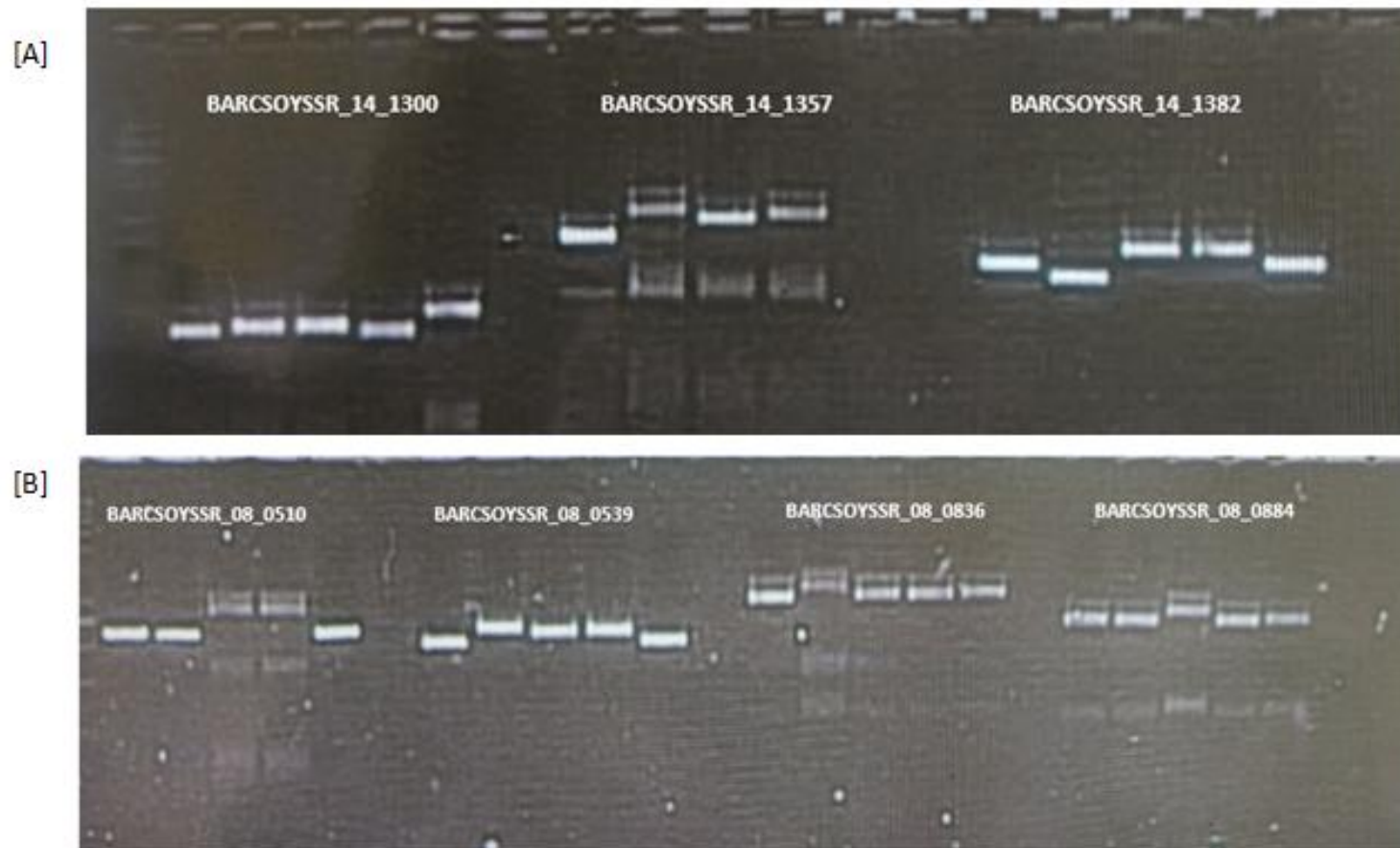


Figure 1: PPS analysis using SSR markers from linkage groups (A) B2 and (B) A2. For each SSR marker, lanes 1–5 (from left to right) represent the amplified products of LJ140, NRC 130, NRC 138, NRC 204, and JS 20-34, respectively.

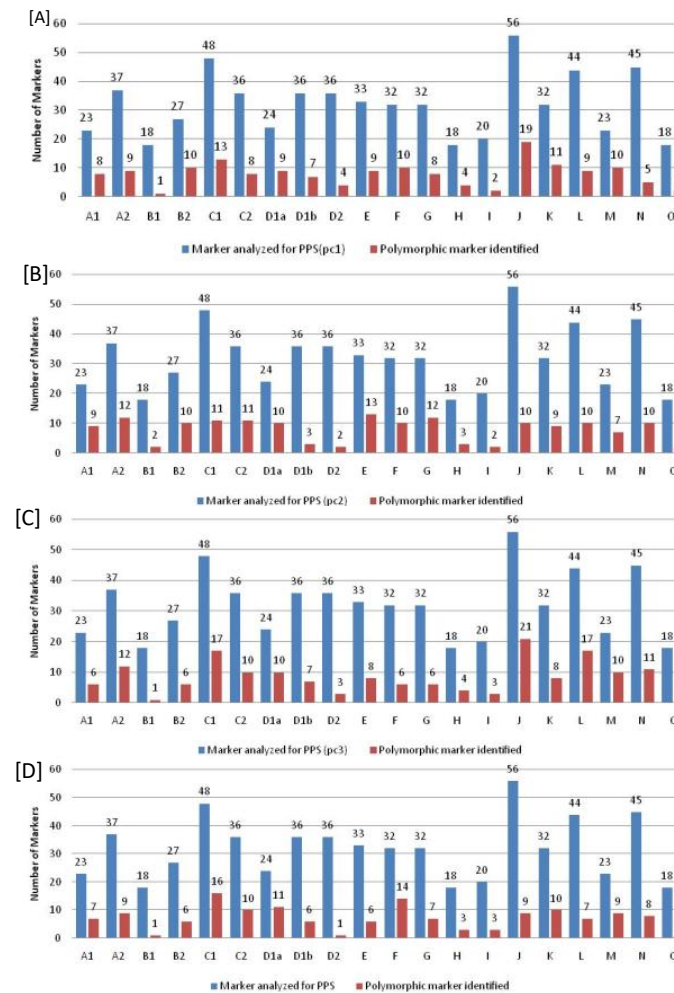


Figure 2: Graphical representation of total number of marker run and total number of polymorphic marker identified in four different parental combinations (A) NRC 130 × LJ 140, (B) NRC 138 × LJ 140, (C) NRC 204 × LJ 140, and (D) JS 20-34 × LJ 140.

Table 3: Percentage Polymorphism across 20 Linkage groups present in different parental combinations (A) NRC 130 × LJ 140, (B) NRC 138 × LJ 140, (C) NRC 204 × LJ 140, and (D) JS 20-34 × LJ 140.

Linkage Group	% Polymorphism(NRC130 X LJ140)	% Polymorphism(NRC 138 X LJ 140)	% Polymorphism(NRC 204 X LJ 140)	% Polymorphism(JS 20-34 X LJ140)
A1	34.78	39.13	26.09	30.43
A2	24.3	32.4	32.4	24.3
B1	5.56	11.11	5.56	5.56
B2	37.04	37.04	22.22	22.22
C1	27.08	22.92	35.42	33.33
C2	22.22	30.56	27.78	27.78
D1a	37.5	41.67	41.67	45.83
D1b	19.44	8.33	19.44	16.67
D2	11.11	5.56	8.33	2.78
E	27.27	39.39	24.24	18.18
F	31.25	31.25	18.75	43.75
G	25	37.5	18.75	21.88
H	22.22	16.67	22.22	16.67
I	10	10	15	15
J	33.92	17.85	37.5	16.07
K	34.38	28.13	25	31.25
L	20.45	22.73	38.64	15.91
M	43.48	30.43	43.48	39.13
N	11.11	22.22	24.44	17.78
O	11.11	11.11	5.56	5.56

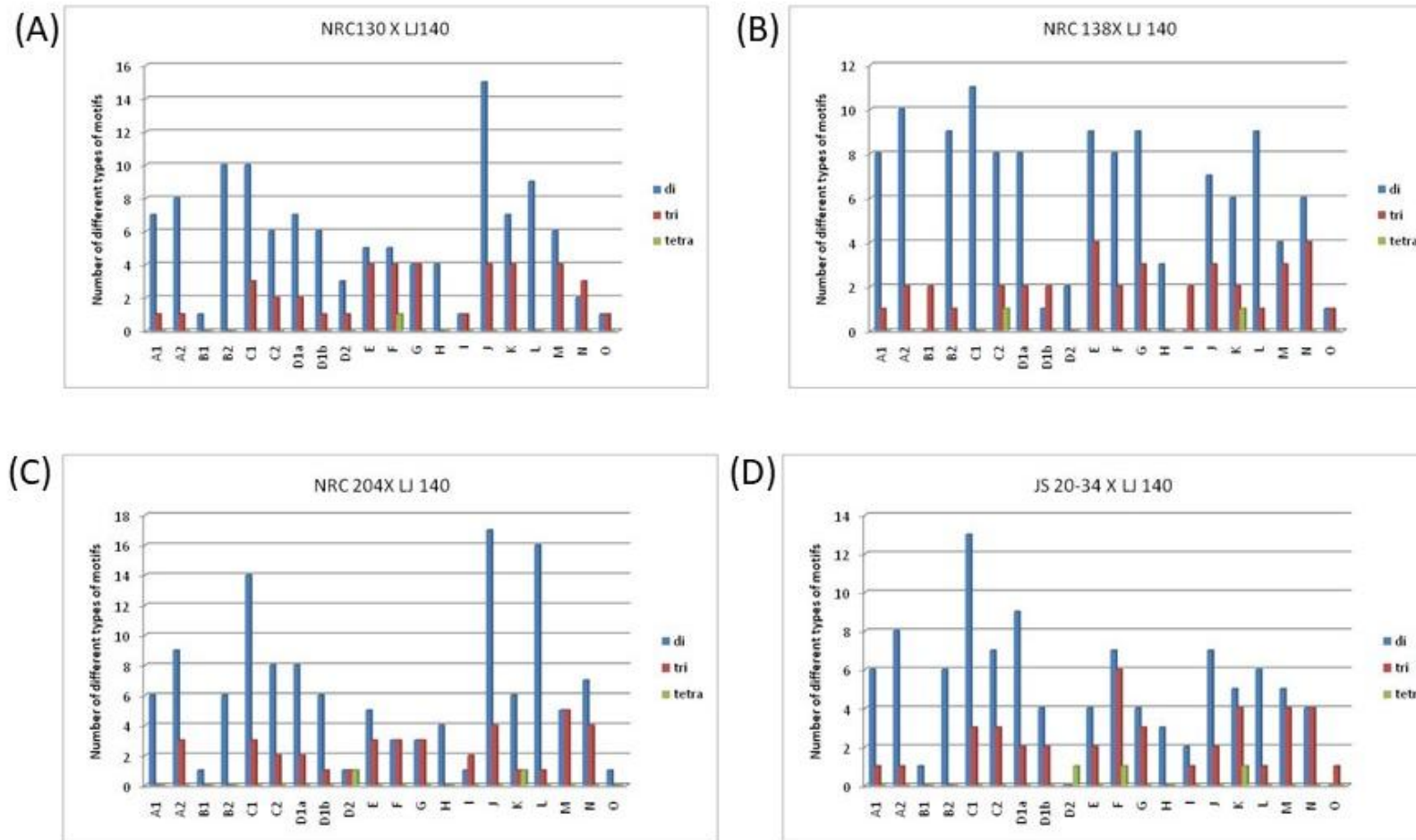


Figure 3: Presence of different types of polymorphic repeat motifs on 20 linkage groups of soybean for different parental combinations (A) NRC 130 × LJ 140, (B) NRC 138 × LJ 140, (C) NRC 204 × LJ 140, and(D) JS 20-34 × LJ 140.

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Management of Defoliators Insect-pests of Soybean by Using Intercrop with Suva, *Anethum graveolens*

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ABSTRACT

*For management of defoliators insect-pests of soybean an experiment was laid in Kharif 2021 and 2022 at the ICAR-Indian Institute of Soybean Research, Indore (Madhya Pradesh) with total five treatments were taken in various rows combinations of suva with soybean including sole soybean crop. The results were revealed that all the combinations have lesser populations of semiloopers and tobacco caterpillar as compare to sole soybean crop in both the years. The lowest population of semiloopers 3.08 and 12.08 larva/meter row length was found in T5 (6 soybean: 2 suva: 6 soybean rows combinations) treatment, respectively whereas the highest population 3.92 and 15.34 larva/meter row length was found in T1 (Sole Soybean 12 rows) treatment, respectively. The mean population of semiloopers (7.83 larva/meter row length) was also found lowest in T5 (6 soybean: 2 suva: 6 soybean rows combinations) treatment. In case of tobacco caterpillar was the lowest population was found in T2 (3 soybean: 1 suva: 3 soybean rows combinations) treatment whereas the highest population was found in T1 (sole soybean 12 rows). The mean population of tobacco caterpillar (7.83 larva/meter row length) was also found lowest in T2 (3 soybean: 1 suva: 3 soybean rows combinations) treatment. The highest grain yield of soybean was observed in T2 (3 soybean: 1 suva: 3 soybean rows combinations) as 2291 and 1492 kg/ha in 2021 and 2022, respectively. The highest average grain yield 1892 kg/ha was also observed in T2 (3 soybean: 1 suva: 3 soybean rows combinations). This study provides information on concept of intercropping of soybean with Suva, *Anethum graveolens* for defoliators insect-pests management.*

Key words: Soybean insect-pests. Defoliators. Suva, *Anethum graveolens*. Intercrop.

Soybean (*Glycine max* Merrill) is one of the most important oil seed crops grown in India especially in Central India viz., Madhya Pradesh, Maharashtra and Rajasthan. It is infested by more than 270 pests species (Patil, Kulkarni and Gavkare, 2014; Kambrekar, Jahagirdar

and Aruna 2017) but only 10-15 pests viz., *Spodoptera litura*, semiloopers (*Dichrysia orichalcea*; *Chrysodeixis acuta*; *Gesonina gemma*; *Mocis undata*, *Obereopsis brevis*, *Melanagromyza sojae*, *Bemisia tabaci*; *Spilosoma* (*Diacrisia*) *oblique* etc.) species are causing the economic loss to

crop (Babu, Meena and Dudwal, 2017). Among these insect-pests defoliator insect-pests are very important especially during reproductive phase of the crop where they are causing more losses (20-30% yield loss) to the crop [Tripathi and Sharma, 1985]. So, it is very important to manage these defoliators. For their management there are several options but applications of different chemical pesticides are most commonly used by farmers. But, these chemicals give effective control but create several problems to the environment such as insecticide resistant among pests, pesticides residues in soil, seeds, air and foods, pest resurgence etc. Due to these problems non chemical methods of pest management are evolved. Intercropping/trap cropping is such a method of pest control in soybean. This study is based on concept of intercropping of soybean with Suva, *Anethum graveolens* for defoliators insect-pests such as semiloopers and tobacco caterpillar management in various rows combinations.

MATERIALS AND METHODS

For management of defoliators insect-pests of soybean an experiment was laid in *Kharif* 2021 and 2022 at the ICAR-Indian Institute of Soybean Research, Indore (Madhya Pradesh). The JS 335 soybean variety was used for growing of crop. All recommended agronomic practices were followed for growing of crop. The distance between rows, plots and replications was 0.45 meter, 1meter and 1.5 meter, respectively. For observations on defoliator insect-pests total number of larvae per meter rows was counted at three

places randomly in each plot. There were total five treatments were taken in various rows combinations of suva with soybean. Soybean was also grown as a sole crop to compare the effects of intercrop with sole crop. The treatments from T2-T5 were of different rows combinations of suva and soybean and T1 was of sole soybean crop treatment. The detail about treatments was given below:

T1- Sole Soybean (12 rows)

T2- Soybean + Suva (3 soybean: 1 suva: 3 soybean rows combinations)

T3- Soybean + Suva (6 soybean: 1 suva: 6 soybean rows combinations)

T4- Soybean + Suva (3 soybean: 2 suva: 3 soybean rows combinations))

T5- Soybean + Suva (6 soybean: 2 suva: 6 soybean rows combinations)

Statistical analysis

Randomized block design with four replications was used for growing of crop. For statistical analysis all the row data were transformed by square root transformation (SQRT).

RESULTS AND DISCUSSION

Effects of intercropping in defoliator pests management of soybean during *Kharif* 2021 and *Kharif* 2022 (Table 1)

The results of different rows combinations of soybean with suva, *Anethum graveolens* revealed that all the combinations have lesser populations of semiloopers and tobacco caterpillar as compare to sole soybean crop in both the years. The lowest population of

semiloopers 3.08 and 12.08 larva/meter row length was found in T5 (6 soybean: 2 suva: 6 soybean rows combinations) treatment in 2021 and 2022, respectively whereas the highest population 3.92 and 15.34 larva/meter row length was found in T1 (Sole Soybean 12 rows) treatment in 2021 and 2022, respectively. The mean population of semiloopers of 2021 and 2022 (7.83 larva/meter row length) was also found lowest in T5 (6 soybean: 2 suva: 6 soybean rows combinations) treatment. In case of tobacco caterpillar the lowest population was found in T2 (3 soybean: 1 suva: 3 soybean rows combinations) treatment in 2021 and 2022 whereas the highest population was found in T1 (sole soybean 12 rows). The mean population of tobacco caterpillar of 2021 and 2022 (7.83 larva/meter row length) was also found lowest in T2 (3 soybean: 1 suva: 3 soybean rows combinations) treatment. The highest grain yield of soybean was observed in T2 (3 soybean: 1

suva: 3 soybean rows combinations) as 2291 and 1492 kg/ha in 2021 and 2022, respectively. The highest average grain yield 1892 kg/ha was also observed in T2 (3 soybean: 1 suva: 3 soybean rows combinations).

Quantity of Insecticide Required for Treating Suva v/s sole Soybean on 1 ha (Table 2)

In sole soybean, Chlorantraniliprole 18.5 SC @ 150 ml/ha and Quinalphos 25 EC @ 1500 ml/ha were required for managing larval populations, whereas in case of different combinations of intercrop, where the population was restricted to *suva* the insecticides requirement was reduced to only 11.6 ml/ha to 37.5 ml/ha and 115.5 to 375.0 ml/ha respectively (Table 2). Considering the total area occupied by *Suva*, effective trapping of defoliators and their management, all the row combinations were found to be highly effective.

Table 1: Management of major defoliators of soybean through intercropping with suva, *Anethum graveolens*

Treatments	Treatment Details	2021		2022		Mean of semilooper population	Mean of tobacco caterpillar	Yield (Kg/ha)	Average yield (Kg/ha)	
		Semiloopers	Tobacco caterpillar	Semiloopers	Tobacco caterpillar					
		(larva/meter row length)	(larva/meter row length)	(larva/meter row length)	(larva/meter row length)					
T1	Sole Soybean (12 rows)	3.92	2	15.34	1.33	9.63	1.67	1632	924	1278
		-2.1	-1.58	-3.98	-1.35					
T2	Soybean + Suva (3 soybean: 1suva: 3 soybean rows combinations)	4.25	1.08	12.67	0.33	8.46	0.71	2291	1492	1892
		-2.18	-1.26	-3.63	-0.91					
T3	Soybean + Suva (6 soybean: 1 suva: 6 soybean rows combinations)	5.17	1.5	12.58	1.25	8.63	1.38	1803	999	1401
		-2.38	-1.41	-3.62	-1.32					
T4	Soybean + Suva (3 soybean: 2 suva: 3 soybean rows combinations)	4.42	1.5	12.5	0.92	8.46	1.21	2169	1257	1713
		-2.22	-1.41	-3.61	-1.19					
T5	Soybean + Suva (6 soybean: 2 suva: 6 soybean rows combinations)	3.08	1.25	12.08	0.67	7.83	0.96	1890	1127	1509
		-1.89	-1.32	-3.55	-1.08					
	CD at 5%	-0.96	-0.74	-0.52	-0.41			457	259	
	SEm±	-0.44	-0.34	-0.24	-0.19			210	119	
	CV	-24.64	-31.76	-7.9	-20.02			13.1	12.5	

*figures in parenthesis are square root transformed values (SQRT)

Economic Feasibility of Trap Cropping with Suva (Table 3)

The expenditure on application of two insecticides in sole soybean and in combinations with *Suva* is given in Table 3. It is evident that expenditure on insecticides required for treating *Suva* rows only was considerable less as compared to treating sole soybean. Least expenditure was incurred in case of 6:1:6 row combination.

This study are closely related to the findings of Das (1998) and, Meena *et al.* (2001) who reported lower incidence of *H. armigera* on chickpea intercropped with wheat, mustard, barley and safflower and

Shivaleela and Nandihalli (2010) also recorded lower incidence of pod borer in chickpea+safflower and chickpea +wheat combination. The present study are also in confirmation with the findings of Yadav *et al.* (2007) who recorded minimum larval population of gram pod borer at peak on chickpea+safflower intercrop combination whereas, the maximum population was found being in chickpea+linseed and chickpea+wheat intercrop combination. Ahmad *et al.* (2016) also recorded lower larval population of *H. armigera* on chickpea+mustard and chickpea+barley intercrop combination which supports the present findings.

Table 2: Quantity of insecticide required for treating Suva v/s sole soybean on 1 ha (Calculated on the basis of experimental area)

Treatment	No. of Suva rows/ha	Chlorantraniliprole	Quinalphos
		(ml/ha)	(ml/ha)
Sole Soybean (12 rows)	--	150	1500
Soybean + Suva (3:1:3)	635	24.1	214.4
Soybean + Suva (6:1:6)	342	11.6	115.5
Soybean + Suva (3:2:3)	1111	37.5	375
Soybean + Suva (6:2:6)	635	24.1	214.4

Table 3: Cost of insecticide required for treating Suva v/s sole soybean

Treatment	No. of Suva rows/ha	Chlorantraniliprole	Quinalphos	Total
		(Rs./ha) #	(Rs./ha) #	(Rs./ha)
Sole Soybean	--	1875	600	2475
Soybean+Suva (3:1:3)	635	301.25	85.76	387.01
Soybean+Suva (6:1:6)	342	145	46	191
Soybean+Suva (3:2:3)	1111	468.75	150	618.75
Soybean+Suva (6:2:6)	635	301.25	85.76	387.01

Chlorantraniliprole @ Rs. 12500/- per lit; Quinalphos @ Rs. 400/- per lit

CONCLUSION

Based on the present research results all the different rows intercrop combinations of soybean with suva *Anethum graveolens* were found superior as compare to control (sole soybean) in having lesser larval populations of both tobacco caterpillar and semiloopers. Based on the required insecticides quantity as well as on the basis of economic feasibility of trap cropping with suva, all the trap crop combinations were found superior as compare to sole soybean cropping. They required lesser area to be treated which ultimately reduced the quantity of insecticide required for control of defoliator pests of soybean. It will be prove fruitful in terms of environmental safety.

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Evaluation of biopesticides against major defoliators infesting soybean

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ABSTRACT

Field trial was conducted in Kharif 2021-22 at AICRP on Soybean, VNMKV, Parbhani (M.S.) to evaluate the bioefficacy of different biopesticides against major defoliators of soybean. Among the different treatments on the basis of larval population per meter row length (mrl), after 2nd spray at 10 DAT (Days after treatment) biopesticides treatment, *N. rileyi* @ 4 ml/l found superior over rest of the microbial agents in reducing the population of semiloopers and no semilooper larvae were observed. However, it was found at par with the microbial agent treatments *B. bassiana* @ 4 ml/l (0.08 larvae/mrl), *Bt* @ 2 ml/l (0.17 larvae/mrl) and *M. anisopliae* @ 4 ml/l (0.25 larvae/mrl). Highest of 1.75 semilooper larvae/mrl were observed in untreated control. For *Spodoptera litura*, after 2nd spray at 10 DAT, no *Spodoptera* larvae were observed in microbial agent treatments *B. bassiana* @ 4 ml/l, *M. anisopliae* @ 4 ml/l, *N. rileyi* @ 4 ml/l and *Bt* @ 2 ml/l. However, highest of 0.33 larvae/mrl were recorded in untreated control. All the microbial agents recorded significantly higher yield than untreated control. The microbial agent treatment *N. rileyi* @ 4 ml/l recorded highest of 1080.37 kg/ha yield. However, it was found at par with microbial agent treatments *B. bassiana* @ 4 ml/l (1045.82 kg/ha), *Bt* @ 2 ml/l (1019.90 kg/ha) and *M. anisopliae* @ 4 ml/l (1011.26 kg/ha). Untreated control recorded significantly lowest yield of 716.95 kg/ha.

Key words: Soybean, Defoliators, Semilooper, *Spodoptera litura*, Biopesticides

Soybean, *Glycine max* (Merill), also known as the “Wonder crop”. It is most important oilseed crop among the pulses and belongs to family *leguminaceae* and sub family *papilionaceae*. The native of soybean is in China (1960) and was introduced in India in 1970-80 (Andole, 1984). Soybean is mainly cultivated in USA, China, Brazil, Argentina and India.

India ranks fourth in world in respect of area and fifth in terms of production (Directors report 2019-20, IISR, Indore). In India in the year 2020-21, soybean cultivation reached to 13.58 million tons from an area of 12.12 million ha and a productivity of 1125 kg/ha. Maharashtra state rank second in area (4.32 m ha)

after Madhya Pradesh (5.85 m ha) (Directors report 2020-21, IISR, Indore).

Recently, soybean is gaining importance because of various quality factors. It is especially interesting to vegetarians because of rich protein content (40%) and edible oil (20%). Soya protein also possess major essential amino acids useful in human nutrition and vitamins B and D. It provided 5 times more protein than cereals and twice that of pulses, 6-7% total minerals, 5-6 % crude fiber and 17-19% carbohydrates (Chauhan and Joshi, 2005). Area under soybean crop is increasing in Maharashtra with a tremendous scope particularly in Vidarbha, South Maharashtra and Marathwada. However, due to severe attack of pests on soybean crop, there is no certainty in harvesting the crop in good condition. The low productivity of soybean both at state and national level is attributed due to biotic and abiotic stresses like drought, weeds, insect pests and diseases (Adimani, 1976). In India, 20 insect pest species have been recorded infesting soybean crop (Singh and Singh, 1990). In Maharashtra, particularly in Marathwada 19 species have been identified attacking this crop (Mundhe, 1982).

The soybean defoliators mainly include tobacco caterpillar (*Spodoptera litura*, Fabricius) and green semilooper (*Chrysodeixis acuta*). Immature stages (larva or caterpillar) of both Tobacco leaf eating caterpillar and green semilooper damages the crop at vegetative stage and in severe cases, it completely defoliates the crop and drastic yield loss (Singh and Singh, 1990). Patel *et al.* (2014) reported that the biopesticides *Bacillus*

thuringiensis (Bt) and *Beauveria bassiana* were found effective against defoliators of soybean. Chaware *et al.* (2019) also reported the importance of various biopesticides viz. *Metarhizium anisopliae*, *Beauveria bassiana* and *Nomuraea rileyi* against soybean defoliators. Ansari and Sharma (2005) suggested that the *Bacillus thuringiensis* and *Beauveria bassiana* with new insecticides recommended for insect pest control in soybean. However, Balikayi *et al.* (2022) reported that combination of biopesticides also proved effective against soybean defoliators. Till today the major emphasis is given on chemical pest management. However, due to indiscriminate use of chemical insecticides to control the pests has led to various problems viz., development of resistance to insecticides, pest resurgence, residues in food grains besides environmental pollution. Kranthi *et al.*, 2002 reported that the pest has developed resistance against a variety of insecticides belonging to almost all the insecticide groups used for the control of pests among crops. Thus, it is an urgent need to advocate ecofriendly insecticides to mitigate the adverse effects of chemical pesticides causing environmental problems. All these undesirable effects of insecticides have led to identification of different substitutes which includes use of botanicals and microbial insecticides like NSKE, Neem oil, *M. anisopliae*, *N. rileyi*, *B. bassiana*, *B. thuringiensis* etc. More than 700 species of fungi, mostly Deuteromycetes and Entomophorales from about 90 genera are pathogenic to insects (Rombach *et al.*, 1986).

Entomopathogens as biocontrol agents offer good and effective alternative to conventional insecticides. Out of which the biopesticides viz. *B. bassiana*, *M. anisopliae*, *N. rileyi*, *B. thuringiensis* have played an important role for the management of various defoliators of soybean. However, its efficacy needs to be tested against changing pest situation in comparison with the chemical insecticides (Anonymous 1989). Considering above facts this study was carried out to evaluate the different biopesticides against major defoliators infesting soybean.

MATERIAL AND METHODS

Field experiment was conducted on soybean during *Kharif* 2021-22 at the Research Farm of All India Coordinated Research Project on Soybean, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (M.S.). The experiment was laid out in a Randomized block design (RBD) with five treatments and four replications for the management of soybean defoliators viz., Semiloopers (*G. gema*, *C. acuta*, *D. orichalcea* etc.) and tobacco leaf eating caterpillar (*Spodoptera litura* (F.)). Four biopesticides viz., *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Bacillus thuringiensis* (Bt) along with untreated control (Table 1). Insect pests susceptible soybean variety MAUS 2 was used for the trial. Plot size was

2.70 x 3.0m (6 rows of 3m length each) with spacing of 45 cm x 5 cm between Rows and Plants, respectively. All the recommended Agronomical practices were followed to raised a good crop. Spraying of foliar biopesticides were administered when the pest population crossed economics threshold level (ETL). Two spray's were undertaken with 10 day's interval. Total numbers of defoliator's larvae per meter row length from three randomly selected places per replication were recorded at before spray, 3, 7 and 10 days after 1st and 2nd spray. The yield data from each treated plot were used to calculate economics of spraying. The cost of entomopathogenic fungi, cost of spray applications i.e. labour charges prevailing during the course of investigations were taken into consideration to work out the cost of each treatment per hectare. Similarly, income obtained from the sale of soybean as per the prevailing market rates was also calculated for each treatment to work out the Incremental cost benefit ratio (ICBR).

Statistical analysis

The field data collected during the course of experimentation were subjected to statistical analysis after appropriate transformation for interpretation of results. Randomized block design (RBD) used in order to test level of significance among the various treatments as per Gomez and Gomez (1984).

Table 1: Biopesticide treatment details

T. N	Treatment details	Concentration	Dose	Formulation	Formulation type	Source of microbial agent
				ml/l water		
1	<i>Beauveria bassiana</i>	1 x 10 ⁸ CFU/ml	2 lit/ha	4 ml/l	Liquid formulation	Dept. of Plant Pathology, VNMKV, Parbhani (MS)
2	<i>Metarhizium anisopliae</i>	1 x 10 ⁸ CFU/ml	2 lit/ha	4 ml/l		
3	<i>Nomuraea rileyi</i>	1 x 10 ⁸ CFU/ml	2 lit/ha	4 ml/l		
4	<i>Bacillus thuringiensis (Bt) commercial</i>	17600 IU/mg	1 lit/ha	2 ml/l	Liquid formulation	Dipel (Sumitomo Chemical India Pvt. Ltd. Gujarat)
5	Untreated control	--	--	--	--	--

RESULTS AND DISCUSSION

Bioefficacy of biopesticides against major defoliators of soybean

A. Semiloopers (*G. gema*, *C. acuta*, *D. orichalcea* etc.):

The data presented in Table 2 revealed that during *Kharif* 2021-22, the overall incidence of semilooper was more. The data indicated that before spray there were no significant differences in larval population of semiloopers (Range 7.92 to 9.08 larvae/mrl) among the treatments. At 3 DAT, microbial agent treatment *N. rileyi* @ 4 ml/l recorded significantly lowest of 6.83 larvae/mrl. However, it was found at par with treatment *B. bassiana* @ 4 ml/l (7.33 larvae/mrl). It was followed by treatments *M. anisopliae* @ 4 ml/l and *Bt* @ 2 ml/l (each, 7.92 larvae/mrl). Highest of 9.42 larvae/mrl were recorded in untreated control. At 7 DAT, microbial agent treatment *N. rileyi* @ 4 ml/l recorded lowest of 3.42 larvae/mrl. Next best microbial agent treatments were *B. bassiana* @ 4 ml/l (4.25 larvae/mrl) and *Bt* @ 2 ml/l (4.92 larvae/mrl) which were found at par with each other. It was followed by biopesticide treatment *M. anisopliae* @ 4 ml/l (5.08 larvae/mrl). Highest semiloopers larvae were observed in untreated control (11.83 larvae/mrl). At 10 DAT, microbial agent treatment *N. rileyi* @ 4 ml/l recorded lowest of 2.83 larvae/mrl. Next best microbial agent treatments were *B. bassiana* @ 4 ml/l (3.17 larvae/mrl) and *Bt* @ 2 ml/l (3.33 larvae/mrl) which were found at par with each other. It was followed by biopesticide treatment *M.*

anisopliae @ 4 ml/l (3.42 larvae/mrl). Highest semiloopers larvae were observed in untreated control (8.17 larvae/mrl).

After 2nd spray, at 3 DAT, more or less similar trend was observed and the microbial agent treatment *N. rileyi* @ 4 ml/l recorded significantly lowest of 1.83 larvae/mrl. However, it was found at par with microbial agent treatment *B. bassiana* @ 4 ml/l (2.17 larvae/mrl). It was followed by microbial agent treatments *Bt* @ 2 ml/l (2.42 larvae/mrl) and *M. anisopliae* @ 4 ml/l (2.50 larvae/mrl) which were found at par with each other. Highest semilooper larvae recorded in untreated control (5.92 larvae/mrl). At 7 DAT, microbial agent treatment *N. rileyi* @ 4 ml/l recorded significantly lowest of 0.75 larvae/mrl and found superior over rest of the microbial agents in reducing the population of semiloopers. However, it was found at par with the microbial agent treatments *B. bassiana* @ 4 ml/l (1.00 larvae/mrl), *Bt* @ 2 ml/l (1.08 larvae/mrl) and *M. anisopliae* @ 4 ml/l (1.17 larvae/mrl). Highest semilooper larvae i.e. 2.92 larvae/mrl were observed in untreated control. At 10 DAT, microbial agent treatment *N. rileyi* @ 4 ml/l found superior over rest of the microbial agents in reducing the population of semiloopers and no semilooper larvae were observed. However, it was found at par with the microbial agent treatments *B. bassiana* @ 4 ml/l (0.08 larvae/mrl), *Bt* @ 2 ml/l (0.17 larvae/mrl) and *M. anisopliae* @ 4 ml/l (0.25 larvae/mrl). Highest semilooper larvae i.e. 1.75

larvae/mrl were observed in untreated control.

The population of semiloopers was naturally decreased during and after 2nd spray due to heavy & continuous rainfall. Thus, according to the above results microbial agent treatment *N. rileyi* @ 4 ml/l was found most effective against the semiloopers, of soybean in comparison with other microbial agents by recording lowest larvae/mrl. However, most of the times it was found at par with microbial agent treatments *B. bassiana* @ 4 ml/l and *Bt* @ 2 ml/l. Similar result were noticed by Patil and Abhilash (2014) where pooled analysis of two years data revealed that *N. rileyi* proved superior over untreated control and at par with other biorationals (*Bt*, NSKE, etc.) in reducing the semilooper larval population. Effectiveness of *B. bassiana* against green semilooper was also reported by Shinde (2011). Dhepe *et al.* (2018) reported the effectiveness of *B. bassiana* and *Bt* against green semilooper (*T. orichalcea*). Effectiveness of *Bt* has been also supported by Somase (2006). Chaware *et al.* (2019) also reported the superiority of entomopathogenic fungi viz., *M. anisopliae*, *B. bassiana* and *N. rileyi* at different concentrations against green semilooper over control which is in confirmation with the results obtained during present investigation.

B. *Spodoptera litura* :

The data presented in Table 3, revealed that during Kharif 2021-22, the overall incidence of *S. litura* was very less. The data indicated that before spray there were no significant differences in the larval population of *Spodoptera* larvae among the treatments. At 3 DAT, biopesticides treatment *N. rileyi* @ 4 ml/l recorded lowest of 0.50 larvae/mrl. However, it was found at par with microbial agent treatments *B. bassiana* @ 4 ml/l (0.67 larvae/mrl) and *Bt* @ 2 ml/l (0.75 larvae/mrl). It was followed by treatment *M. anisopliae* @ 4 ml/l (0.92 larvae/mrl). Highest of 1.17 *Spodoptera* larvae/mrl were recorded in untreated control. At 7 DAT, lowest of 0.25 larvae/mrl were recorded in biopesticide treatment *N. rileyi* @ 4 ml/l. However, it was found at par with microbial agent treatments *B. bassiana* @ 4 ml/l (0.33 larvae/mrl), *Bt* @ 2 ml/l (0.42 larvae/mrl) and *M. anisopliae* @ 4 ml/l (0.50 larvae/mrl). Highest *Spodoptera* larvae were recorded in untreated control (1.25 larvae/mrl). At 10 DAT, microbial agent treatment *N. rileyi* @ 4 ml/l recorded lowest of 0.08 larvae/mrl. Next best microbial agent treatments were *B. bassiana* @ 4 ml/l (0.17 larvae/mrl) and *Bt* @ 2 ml/l (0.25 larvae/mrl). It was followed by biopesticide treatment *M. anisopliae* @ 4 ml/l (0.42 larvae/mrl). Highest *Spodoptera* larvae were observed in untreated control (1.08 larvae/mrl).

Table 2: Effect of microbial agents on population of semiloopers infesting soybean

T. N.	Dose / lit		1 st spray				2 nd spray			
	Treatment details		DBT	3 DAT	7 DAT	10 DAT	DBT	3 DAT	7 DAT	10 DAT
1	<i>B. bassiana</i>	4 ml	7.92 -2.89	7.33 -2.79	4.25 -2.17	3.17 -1.91	3.17 -1.91	2.17 -1.62	1 -1.22	0.08 -0.75
2	<i>M. anisopliae</i>	4 ml	8.25 -2.95	7.92 -2.9	5.08 -2.36	3.42 -1.97	3.42 -1.97	2.5 -1.72	1.17 -1.28	0.25 -0.86
3	<i>N. rileyi</i>	4 ml	8.33 -2.97	6.83 -2.7	3.42 -1.97	2.83 -1.82	2.83 -1.82	1.83 -1.52	0.75 -1.11	0 -0.7
4	<i>Bacillus thuringiensis (Bt)</i>	2 ml	9.08 -3.09	7.92 -2.9	4.92 -2.32	3.33 -1.95	3.33 -1.95	2.42 -1.7	1.08 -1.25	0.17 -0.8
5	Untreated Control	--	8.25 -2.95	9.42 -3.14	11.83 -3.51	8.17 -2.94	8.17 -2.94	5.92 -2.53	2.92 -1.84	1.75 -1.49
	S.E. ±		0.04	0.05	0.05	0.04	0.04	0.05	0.07	0.05
	C.D. at 5%		NS	0.16	0.16	0.14	0.14	0.17	0.22	0.16

Figures in parentheses are $\sqrt{x + 0.5}$ transformed values, DBT = Day before treatment, DAT = Days after treatment, NS = Non significant.

After 2nd spray, at 3 DAT, no *Spodoptera* larvae was observed in microbial agent treatment *N. rileyi* @ 4 ml/l. However, it was found at par with microbial agent treatments *B. bassiana* @ 4 ml/l (0.08 larvae/mrl) and *Bt* @ 2 ml/l (0.17 larvae/mrl). It was followed by biopesticide treatment *M. anisopliae* @ 4 ml/l (0.33 larvae/mrl). Highest *Spodoptera* larvae were recorded in untreated control (0.75 larvae/mrl). At 7 DAT, there was higher reduction in larval population and no *Spodoptera* larvae were observed in microbial agents treatments *B. bassiana* @ 4 ml/l, *N. rileyi* @ 4ml/l and *Bt* @ 2 ml/l however, these treatments were found at par with microbial agents treatments *M. anisopliae* @ 4 ml/l (0.08 larvae/mrl). Highest of 0.50 larvae/mrl were recorded in untreated control. At 10 DAT, no *Spodoptera* larvae were observed in microbial agents treatments *B. bassiana* @ 4 ml/l, *M. anisopliae* @ 4 ml/l, *N. rileyi* @ 4 ml/l and *Bt* @ 2 ml/l. Highest of 0.33 larvae/mrl were recorded in untreated control.

The population of *Spodoptera* larvae was naturally decreased during and after 2nd spray due to heavy and continuous rainfall. Thus, according to the above results microbial agents treatments *N. rileyi* @ 4 ml/l was found most effective against the *S.litura* of soybean in comparison with other microbial agents by recording lowest larvae/mrl. However, most of the times it was found at par with microbial agent treatments *B. bassiana* @ 4 ml/l and *Bt* @ 2 ml/l. Similar findings of highly effectiveness of entomopathogenic fungi

N. rileyi against *S. litura* reported by Ambethgar *et al.* (1998), Patil & Hegde (2009), Bade *et al.* (2014) and Swathi *et al.* (2018) which is in agreement with present findings. Patil and Abhilash (2014) reported that the higher dose (2×10^8 conidia/l) of *N. rileyi* proved superior over untreated control and lower dose (1×10^8 conidia/l) of micopathogen and also found on par with other biorationals viz., *Bt*, *SINPV* and *NSKE* 5 per cent against caterpillars of soybean infesting soybean which is in corroboration with present findings. Chaware *et al.* (2019) also reported the superiority of entomopathogenic fungi viz., *M. anisopliae*, *B. bassiana*, and *N. rileyi* at different doses (1×10^8 CFU/ml & 1×10^9 CFU/ml) against tobacco leaf eating caterpillar which is in confirmation with the results obtained during present investigation using different microbial control agent. Purwar and Yadav (2003) also reported the effectiveness of entomopathogenic fungi *B. bassiana* than botanicals against *S. litura* in soybean. In present finding the effectiveness of *Bt* was found to be at par with the microbial agent *N. rileyi*. Dhepe *et al.* (2018) reported that among the different microbial biopesticide treatments *B. bassiana* 5g/l was found to be the most effective as it recorded the highest larval reduction population followed by *B. bassiana* 4g/l., *Bt*. 3g/l, *N. rileyi* 6g/l, *Bt*. 2g/l and *M. anisopliae* 4g/l which is in corroboration with the present finding. These finding are in corroboration with the findings reported by Lalitha *et al.* (2012) against *S. litura* in groundnut. Chaudhari *et al.* (2020) found the effectiveness of *Bt* Var. Kurstaki 0.5 per

cent WP @ 500 g/ha for controlling the tobacco leaf eating caterpillar (*S. liura*) population infesting soybean crop.

Yield:

The data presented in Table 3 revealed that all the microbial agents recorded significantly higher yield than untreated control. The microbial agent treatment *N. rileyi* @ 4 ml/l recorded highest of 1080.37 kg/ha yield. However, it was found at par with microbial agents treatments *B. bassiana* @ 4 ml/l (1045.82 kg/ha), *Bt* @ 2 ml/l (1019.90 kg/ha) and *M. anisopliae* @ 4 ml/l (1011.26 kg/ha). Untreated control recorded significantly lowest yield of 716.95 kg/ha. Patil and Abhilash (2014) reported the higher yield of *N. rileyi* (1×10^8 conidia/l) 1310 kg/ha, *N. rileyi* (2×10^8 conidia/l) 1605 kg/ha and *Bt* (1 ml/l) 1530 kg/ha over control 1160 kg/ha. This supports the present finding. Similar findings of higher yield of biopesticides viz., *B. bassiana*, *M. anisopliae*, *N. rileyi*, over control was reported by Dhepe *et al.* (2018) and Chaware *et al.* (2019) which is in agreement with present findings. In

present investigation microbial agent treatment *Bt* recorded (1019.90 kg/ha) seed yield which is corroboration with the findings of Chaudhari *et al.* (2020) where they recorded 1800 kg/ha yield in *Bt*.

Economics (ICBR):

As regards monetary returns (Table 4), maximum monetary returns were obtained from the microbial agent treatment *N. rileyi* (Rs. 14355/-) followed by *B. bassiana* @ 4 ml/l (Rs. 12990/-), *Bt* @ 2 ml/l (Rs. 11966/-) and *M. anisopliae* @ 4 ml/l (Rs. 11625/-). In case of ICBR, the higher ICBR was obtained from the microbial treatment *N. rileyi* @ 4 ml/l (1:5.31) followed by microbial treatments *B. bassiana* @ 4 ml/l (1:4.81), *M. anisopliae* @ 4 ml/l (1:4.30) and *Bt* @ 2 ml/l (1:2.15). The present findings are in agreement with Dhepe *et al.* (2018) and Chaware *et al.* (2019) which reported the increased yield and highest incremental cost benefit ratio (ICBR) of microbial biopesticides viz., *B. bassiana*, *M. anisopliae*, *N. rileyi* and *Bt*.

Table 3: Effect of microbial agents on population of tobacco caterpillar infesting soybean

T.N.	1 st spray						2 nd spray				
	Treatment details	Dose/ lit	DBT	3 DAT	7 DAT	10 DAT	DBT	3 DAT	7 DAT	10 DAT	Yield kg / ha
1	<i>B. bassiana</i>	4 ml	0.92 -1.18	0.67 -1.07	0.33 -0.9	0.17 -0.8	0.17 -0.8	0.08 -0.75	0 -0.7	0 -0.7	1045.82
2	<i>M. anisopliae</i>	4 ml	1.08 -1.25	0.92 -1.18	0.5 -0.99	0.42 -0.95	0.42 -0.95	0.33 -0.9	0.08 -0.75	0 -0.7	1011.26
3	<i>N. rileyi</i>	4 ml	1 -1.22	0.5 -9.96	0.25 -0.86	0.08 -0.75	0.08 -0.75	0 -0.7	0 -0.7	0 -0.7	1080.37
4	<i>Bacillus thuringiensis (Bt)</i>	2 ml	0.83 -1.15	0.75 -1.11	0.42 -0.95	0.25 -0.85	0.25 -0.85	0.17 -0.8	0 -0.7	0 -0.7	1019.9
5	Untreated	--	1	1.17	1.25	1.08	1.08	0.75	0.5	0.33	
	Control		-1.22	-1.28	-1.32	-1.25	-1.25	-1.11	-0.99	-0.9	716.95
	S.E. ±		0.05	0.05	0.05	0.07	0.07	0.05	0.03	0.03	46.95
	C.D. at 5%		NS	0.17	0.14	0.21	0.21	0.16	0.09	0.1	144.45

Figures in parentheses are $\sqrt{x + 0.5}$ transformed values, DBT = Day before treatment, DAT = Days after treatment, NS = Non significant.

Table 4. Economics of different microbial treatments in soybean

T. N.	Treatment details	Dose	Seed yield (kg/ha)	Increasing in yield over control (kg)	Additional income due to increase in yield (Rs.)	Cost of insecticides including spraying charges (Rs.)	ICBR
1	<i>B. bassiana</i>	2 lit/ha	1045.82	328.87	12990	2700	01:04.8
2	<i>M. anisopliae</i>	2 lit/ha	1011.26	294.31	11625	2700	01:04.3
3	<i>N. rileyi</i>	2 lit/ha	1080.37	363.42	14355	2700	01:05.3
4	<i>Bacillus thuringiensis (Bt)</i>	1 lit/ha	1019.9	302.95	11966	5540	01:02.2
5	Untreated Control	--	716.95	--	--	--	--

Rates:

1. *B. bassiana* : Rs. 150/lit
2. *M. anisopliae* : Rs. 150/lit
3. *N. rileyi* : : Rs. 150/lit
4. *Bt.* (Commercial) : Rs. 1720/lit
5. Spraying charges : Rs. 1050/spraying/ha
6. MSP of soybean (2021) : Rs. 3950/ql

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Phylogenetic and evolutionary analyses of soybean cyclophilins

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ABSTRACT

This study examines the orthologous relationships between cyclophilins in Glycine max (soybean) and Arabidopsis thaliana, with a focus on their evolutionary conservation and functional roles in stress responses. Previously reported GmCYP proteins were analyzed, revealing several orthologs to Arabidopsis cyclophilins associated with light, heat, drought, and pathogen stress. Notably, orthologs of Arabidopsis cyclophilins involved in light-induced oxidative stress (ATCYP20-3, ATCYP20-2), pathogen defense (ATCYP57), and drought tolerance (ATCYP19-1, ATCYP18-2, ATCYP21-2) were identified in G. max, suggesting functional conservation in stress response pathways. Furthermore, the orthology between Arabidopsis ATCYP18-1 and G. max GmCYP42 supported a conserved role in heat stress tolerance. These findings highlighted the evolutionary conservation of cyclophilins between G. max and Arabidopsis. They underscored their potential roles in enhancing stress tolerance through gene duplication and functional diversification.

Keywords: Cyclophilins, soybean, drought, pathogen defense

Cyclophilins are a highly conserved family of proteins found in a wide range of organisms, including bacteria, fungi, higher plants, and animals. They are a subset of immunophilins, which are proteins that interact with immunosuppressive drugs like cyclosporin A, FK506, and rapamycin. These drugs block the activation of T-cells by inhibiting the nuclear translocation of nuclear factor of activated T-cells (NFAT), preventing the expression of cytokines such as interleukin-2, interleukin-4, and

interferon (Göthel & Marahiel, 1999). Cyclophilins specifically bind to cyclosporin A and demonstrate peptidyl-prolyl isomerase activity, catalyzing the cis-to-trans isomerization of proline peptide bonds. This function is essential for protein folding, which positions cyclophilins as molecular chaperones in various cellular processes (Handschumacher et al., 1984; Trandinh et al., 1992; Chou et al., 1997).

Over recent years, genome-wide surveys have provided significant insights into the diversity and distribution of

cyclophilins in plants. For instance, *Brassica napus* has been reported to contain the largest number of cyclophilins (94) with 79 single-domain and 12 multi-domain cyclophilins (Hanhart et al. 2017). In *Triticum aestivum*, 85 cyclophilins have been identified, predominantly localized in the cytoplasm, with 27 being single-domain and 58 multi-domains (Singh et al 2019). Cyclophilins have been widely studied in various plant species, demonstrating their crucial roles across different plant lineages. For instance, *Arabidopsis thaliana* contains 31 cyclophilins (Trivedi et al 2012), while *Gossypium barbadense* has 75, *Gossypium hirsutum* has 79, *Gossypium arboreum* has 40, and *Gossypium raimondii* contains 38 (Chen et al 2019). Other species like *Solanum lycopersicum* have 35 cyclophilins (Khatun et al 2022), *Medicago truncatula* has 33 (Ge et al., 2020), *Malus domestica* contains 30 (Qiao et. al 2022), *Oryza sativa* has 29 (Trivedi et al 2019), *Phaseolus vulgaris* has 26, *Vitis vinifera* has 22, *Cucumis sativus* has 21 (Yadav et. al 2025) and *Glycine max* possesses 62 (Mainali et al 2014). These findings highlight the diverse roles of cyclophilins in processes such as stress response, protein folding, and cellular regulation across a wide range of plant species highlighting the widespread presence and evolutionary diversification of cyclophilins across plant species. Lesser-function-based studies on important proteins like cyclophilins often focus on their roles beyond the well-established functions, such as in protein folding and

immune response. Recent research has suggested that cyclophilins may participate in regulating plant responses to abiotic stresses like drought and heat, modulating cellular redox balance, or influencing gene expression in a way that was previously underappreciated (Kim et al., 2013; Lee et al 2015; Park et al 2015; Romano et al 2004). The present study investigates these less-explored functional roles in abiotic stress through orthologues and similarity studies can which could reveal new facets of protein function that may be crucial for understanding complex biological phenomena and could lead to novel applications in agriculture.

MATERIAL AND METHOD

Sequence Alignment and Phylogenetic Analysis

The protein sequences of previously identified cyclophilins from *G. max*, *A. thaliana* and *P. vulgaris* cyclophilins were aligned using BioEdit version 7.2 (<https://bioedit.software.informer.com>) to assess sequence similarities and evolutionary relationships (Hall et. al 1999). The alignment was subsequently used to construct a phylogenetic tree using the neighbor-joining (NJ) clustering method, and the tree was visualized using Interactive Tree of Life (iTOL) (<https://itol.embl.de/itol.cgi>) (Letunic et. al 2021). This analysis provided insights into the phylogenetic relationships of the cyclophilins identified in *Glycine max* and their orthologues in *Arabidopsis*.

RESULT

Orthologous Relationships Between *Glycine max* Cyclophilins and *Arabidopsis* Cyclophilins

Multiple sequence alignment utilizing clustal W revealed presence of multiple conserved regions in *G. max* and *A. thaliana* where the prominent ones were GRIV, ENFRALTCGE, SIY, LSMAN, TNGSQFFI and HVVFC (Fig.1). In this study, the orthologous relationships between previously reported cyclophilins from *Glycine max* (soybean) and *Arabidopsis thaliana* were explored to uncover their evolutionary conservation and potential functional roles in stress responses (Fig.2). A total of 62 GmCYPs were analyzed, and several key orthologous relationships were identified with *Arabidopsis* cyclophilins, particularly those associated with light, heat, drought, and pathogen stress.

The *Arabidopsis* cyclophilin AT3G62030 (ATCYP20-3), known for its involvement in light-induced oxidative stress (Dominguez-Solis et al., 2008), was found to have orthologues in *Glycine max*, including GmCYP28, GmCYP14, and GmCYP12. Similarly, AT5G13120 (ATCYP20-2), another *Arabidopsis*

cyclophilin involved in light stress, was found to be orthologous to GmCYP25 and GmCYP26, suggesting functional conservation in light-induced stress responses across species (Romano et al., 2004). The *Arabidopsis* cyclophilin AT4G33060 (ATCYP57), which is known for the plant immune response to *Pseudomonas syringae* infection (Pogorelko et al., 2014), was orthologous with GmCYP37 in *Glycine max*. Moreover, *Arabidopsis* cyclophilins AT2G16600 (ATCYP19-1), AT2G36130 (ATCYP18-2), and AT3G55920 (ATCYP21-2), which were upregulated under drought stress and contribute to enhanced stress tolerance (Park et al., 2013; Lee et al., 2015; Liu et al., 2021), were found to be orthologous to GmCYP13, GmCYP15, and GmCYP8 in *Glycine max*. These results suggested that GmCYP13, GmCYP15, and GmCYP8 might play roles in drought tolerance, similar to their *Arabidopsis* counterparts.

Additionally, AT1G01940 (ATCYP18-1), which conferred tolerance to heat stress in *Arabidopsis* (Sakuma et al., 2006), was found to be orthologous to GmCYP42 in *Glycine max*. This orthology suggested that GmCYP42 might also be involved in heat stress tolerance in soybean, similar to its role in *Arabidopsis*.



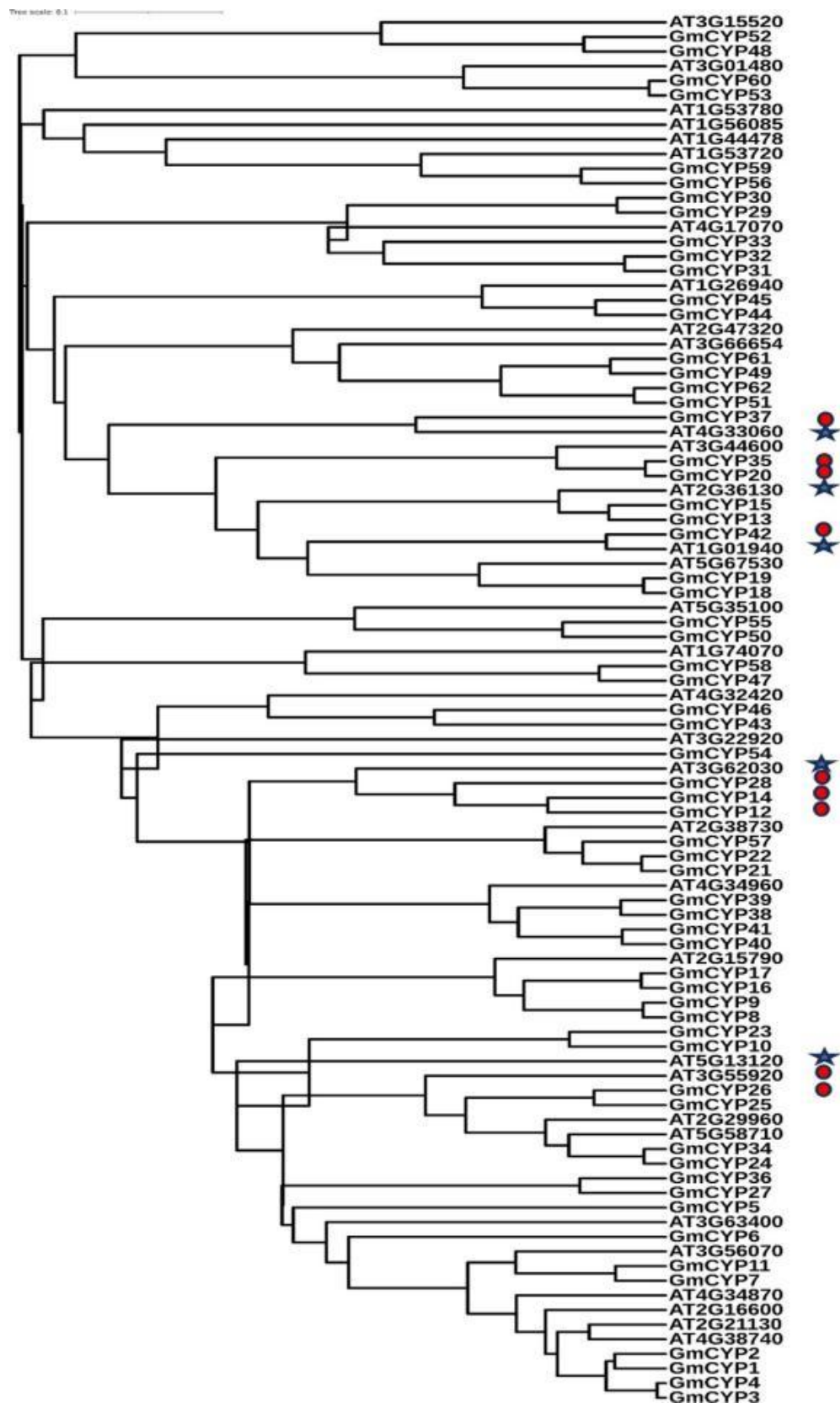


Fig.2: *Glycine max* cyclophilins with their *Arabidopsis* orthologous. *Important *AtCYPs* with functional role in stress tolerance were denoted with star mark whereas circle indicated corresponding *GmCYPs* orthologous.

Comparative analysis of cyclophilins from *Glycine max* and *Phaseolus vulgaris*

The data from a previously published study on *G. max* cyclophilins (Mainali et al., 2014) and our recently published data on *P. vulgaris* cyclophilins (Yadav et al., 2025) were utilized for comparative analysis.

Amino Acid Length:

In *G. max*, the cyclophilins vary in size, with protein lengths ranging from 117 to 850 amino acids. The soybean cyclophilins are generally shorter compared to the cyclophilins of *P. vulgaris*, where the longest cyclophilin, PhvCYP5, contains 933 amino acids, and the shortest, PhvCYP14, has only 136 amino acids. In contrast, soybean cyclophilins, such as GmCYP5, are shorter on average, but both species show considerable variation in size.

Domain Composition:

Both species feature cyclophilins with a mix of single-domain and multi-domain proteins. In *G. max*, many cyclophilins (such as GmCYP1, GmCYP2, GmCYP5, GmCYP12, and others) are single-domain proteins. Similar to soybean, *P. vulgaris* contains several single-domain cyclophilins (e.g., PhvCYP1, PhvCYP2, PhvCYP3, PhvCYP6, and others). However, *P. vulgaris* cyclophilins are more diverse in terms of multi-domain proteins. For instance, PhvCYP5 contains the PHA3307 domain, PhvCYP8 includes both a RAM signaling pathway protein domain and a microneme domain,

and PhvCYP12 features two pairs of ring_Ubox domains.

Subcellular Localization:

The subcellular localization of cyclophilins in both species reveals distinct patterns. In *G. max*, cyclophilins are primarily found in the cytosol, secretory pathway, chloroplasts, mitochondria, and nucleus, reflecting their broad functional diversity across cellular compartments. Similarly, in *P. vulgaris*, most cyclophilins are located in the cytosol (eight), followed by the chloroplast (seven), with one each in the vacuole and mitochondria.

Comparison of Domain Diversity:

While both species have cyclophilins with single domains, *P. vulgaris* shows a greater prevalence of multi-domain cyclophilins. For example, PhvCYP5 includes PHA3307 as an additional domain, and PhvCYP8 possesses both the RAM signaling pathway protein and microneme domains, indicating a more specialized functional complexity in *P. vulgaris*. *G. max* cyclophilins, such as GmCYP5, generally contain a single CLD domain, and fewer multi-domain proteins are present compared to *P. vulgaris*.

Chromosomal Distribution:

The cyclophilins in *Phaseolus vulgaris* are distributed across nine chromosomes, with chromosome 1 containing the highest number (seven cyclophilins). Chromosomes 3 and 4 contain four and three cyclophilins, respectively, and other

chromosomes carry fewer cyclophilins. In comparison, the chromosomal distribution of cyclophilins in *G. max* follows a similar pattern but with differences in exact numbers and chromosome-specific distribution, reflecting the species-specific evolutionary expansion of the cyclophilin family.

Orthologous Study and Multiple Sequence Alignment:

The comparative analysis of cyclophilins between *G. max* and *P. vulgaris* reveals their orthologous relationships (Fig. 3). PhvCYP19 is orthologous to GmCYP50 and GmCYP55, while PhvCYP17 corresponds to GmCYP47 and GmCYP58. PhvCYP8 and PhvCYP5 are orthologous to GmCYP54, and PhvCYP7 aligns with GmCYP14. PhvCYP23 shares homology with GmCYP22 and GmCYP21, and PhvCYP26 is related to GmCYP39 and GmCYP38. PhvCYP2 is orthologous to GmCYP23 and GmCYP10, and PhvCYP18 corresponds to GmCYP9 and GmCYP8. PhvCYP15 aligns with GmCYP16 and GmCYP17,

and PhvCYP4 is orthologous to GmCYP36 and GmCYP27. PhvCYP9 corresponds to GmCYP25 and GmCYP26, and PhvCYP10 shares homology with GmCYP34 and GmCYP24. PhvCYP11 is orthologous to GmCYP7 and GmCYP11, while PhvCYP25 aligns with GmCYP1 and GmCYP2. PhvCYP13 corresponds to GmCYP44 and GmCYP45, and PhvCYP16 shares homology with GmCYP49 and GmCYP61. PhvCYP1 is orthologous to GmCYP62 and GmCYP51, and PhvCYP24 aligns with GmCYP59 and GmCYP56. PhvCYP3 is related to GmCYP37, while PhvCYP6 shares homology with GmCYP15 and GmCYP13. PhvCYP22 is orthologous to GmCYP35 and GmCYP20, and PhvCYP14 corresponds to GmCYP42. Finally, PhvCYP12 is orthologous to GmCYP19 and GmCYP18.

Meanwhile multiple sequence alignment utilizing Clustal W reveals presence of multiple conserved regions in both the plant species where the prominent ones are GRIV, ENFRAICTG, FHR, QGGD, SMAN, TNGSQFFI and HVVF (Fig.4).



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1 .....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....
GmCYP1 1 -----MPNPKVFEDMTIGGQAGRIVMELADVTPTAENFRALCTGKGSFHRVIPSFMCGGDFAGNCTGGESIYG-AKADENFVKHTCPGLLSMANAGEGTNGSQFFICTEKTW
GmCYP2 1 -----MPNPKVFEDMTIGGQAGRIVMELADVTPTAENFRALCTGKGSFHRVIPSFMCGGDFAGNCTGGESIYG-AKADENFVKHTCPGLLSMANAGEGTNGSQFFICTEKTW
GmCYP3 1 -----MPNPKVFEDMTIGGQAGRIVMELADVTPTAENFRALCTGKGAFFHRVIPSFMCGGDFAGNCTGGESIYG-SKADENFIKHTCPGLLSMANAGEGTNGSQFFICTVKTW
GmCYP4 1 -----MANPKVFEDMTIGGQAGRIVMELADVTPTAENFRALCTGKGFHRVIPSFMCGGDFAGNCTGGESIYG-SKADENFIKHTCPGLLSMANAGEGTNGSQFFICTVKTW
GmCYP5 1 -----MPNPKVFEDMTIDGKPVGRIVMGLATNVTPTAENFRALCTGKGSFHRVIPSFMCGGDFAGNCTGGESIYG-KRGS---IYG-AKET---VKKHICLDLSMANIDPETIILKFFICMEKTW
GmCYP6 1 -----MPHPKVFEDMTIGGQVGRIVMELMANTPTAENFRALCTGKGSFHRVIPSFMCGGDFAGNCTGGESIYG-AKGA---VKKHTCPNLSMANASEITNGSQFFICTAEKTKW
GmCYP7 1 -----MSKNPKVFEDMTIGKMKAGRIVMELADVTPTAENFRALCTGKGAFFHRVIPSFMCGGDFAGNCTGGESIYG-SKADENFNLKHTCPGLLSMANAGEGTNGSQFFICTAKTPW
GmCYP8 1 -----MVNPRCFEDVSTIGGELEGRIVVELHDVVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-AKEDENLEVKHERKGLLSMANAGENTNGSQFFITTRTPH
GmCYP9 1 -----MVNPRCFEDVSTIGGELEGRIVVELHDVVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-AKEDENLEVKHERKGLLSMANAGENTNGSQFFITTRTPH
GmCYP10 1 -----AQSKVTQVFEDVSTIGGKLVGRIVIGLGDVVPKTAENFRALCTGKGFHRVIPSFMCGGDFAGNCTGGESIYG-RTEKDNFNLKHTCPGLLSMANAGENTNGSQFFICTVKTW
GmCYP11 1 -----MSKNPKVFEDMTIGKMKAGRIVMELADVTPTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-SKEDENFNLKHTCPGLLSMANAGENTNGSQFFICTVKTW
GmCYP12 1 -----SCFYVEVGGEPVGRIVIGLGEVVPKTVQNEFALYTDYKGSFHRVIPSFMCGGDFAGNCTGGESIYG---FQH-FLAVKHVPGGLLSMANAGENTNGSQFFICTVKTW
GmCYP13 1 -----MWAS-AEGGAPVETLE-TSMGSFTVELYKHAPRTCRNFIELSRR---KFRHRIKDFIVQGGDFAGNCTGGESIYG-AKFEDEIKRELKHTCPGLLSMANAGENTNGSQFFITLAPCPS
GmCYP14 1 -----LQAKVTTKCFEDVSTIGGEPVGRIVIGLGEVVPKTVENFRALCTGKGSFHRVIPSFMCGGDFAGNCTGGESIYG-PSKDESFALKHVPGGLLSMANAGENTNGSQFFICTVKTW
GmCYP15 1 -----MWASAAEGGAPVETLE-TSMGSFTVELYKHAPRTCRNFIELSRR---KFRHRIKDFIVQGGDFAGNCTGGESIYG-AKFEDEIKRELKHTCPGLLSMANAGENTNGSQFFITLAPCPS
GmCYP16 1 -----MGRGRCFEDVSTIGGELEGRIVVELHDVVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-LKEDENFELKHERKGLLSMANAGENTNGSQFFITTRTSH
GmCYP17 1 -----MGRGRCFEDVSTIGGELEGRIVVELHDVVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-LKEDENFELKHERKGLLSMANAGENTNGSQFFITTRTSH
GmCYP18 1 MGKKQHSKDRMFIVKVEKNPKKKGYVQH-H-TTHGDNIELHCDIAPRACENFILTGER---AFHRNIRNFMIQGGDFAGNCTGGESIYG-KPFKDELSKLVHSCRGVLSMANAGENTNGSQFFILYKSANH
GmCYP19 1 MGKKQHSKDRMFIVKVEKNPKKKGYVQH-H-TTHGDNIELHCDIAPRACENFILTGER---AFHRNIRNFMIQGGDFAGNCTGGESIYG-KPFKDELSKLVHSCRGVLSMANAGENTNGSQFFILYKSANH
GmCYP20 1 -----TGSADGHLKFKKKPIFAKHFERSHGPIDGAVSIDGLLCTIENMMVPIPGAVDVVYKQGDVKAADRNSFVHYDIDHMGPIKVMKYNPVCDSVLSADAKGLIETWSPATIQPEEVNI
GmCYP21 1 -----PPNPKNPIVFDVSTIGNIPAGRIKVELADVTAPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-LKEDENFNAKHTCPGLLSMANAGENTNGSQFFITCAKCDW
GmCYP22 1 -----PPNPKNPIVFDVSTIGNIPAGRIKVELADVTAPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-LKEDENFNAKHTCPGLLSMANAGENTNGSQFFITCAKCDW
GmCYP23 1 -----VQSKVTQVFEDVSTIGGKLVGRIVIGLGDVVPKTAENFRALCTGKGFHRVIPSFMCGGDFAGNCTGGESIYG-EKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
GmCYP24 1 -----DLKEVTHKVFEDVSTIGKEAGRIVMGLGKAVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-DKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
GmCYP25 1 -----DLEGVTHKVFEDVSTIGHGKLAGRIVMGLGNTVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-DKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
GmCYP26 1 -----DLEGVTHKVFEDVSTIGHGKLAGRIVMGLGNTVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-DKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
GmCYP27 1 -----MGLEKNPIVFDVSTIGDGPVERIVIQLEASVPTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-GKEDENFNLKHTCPGLLSMANAGENTNGSQFFITFRQPH
GmCYP28 1 -----LQAKVTNKCFFEDVSTIGGEPVGRIVIGLGEVVPKTVENFRALCTGKGSFHRVIPSFMCGGDFAGNCTGGESIYG-GKADENFNLKHTCPGLLSMANAGENTNGSQFFITFRQPH
PhvCYP4 1 -----MGLEKNPIVFDVSTIGDGPVERIVIQLEASVPTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-GKADENFNLKHTCPGLLSMANAGENTNGSQFFITFRQPH
PhvCYP5 1 -----MAKKKNSLVFMDVSTIGDGPVERIVIQLEASVPTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-SKEDENFNLKHTCPGLLSMANAGENTNGSQFFITLAPCPS
PhvCYP6 1 -----MWAS-AEGGAPVETLE-TSMGSFTVELYKHAPRTCRNFIELSRRGYKGSFHRVIPSFMCGGDFAGNCTGGESIYG-PSKDESFALKHVPGGLLSMANAGENTNGSQFFICTVKTW
PhvCYP7 1 -----LQAKVTTKCFEDVSTIGGEPVGRIVIGLGEVVPKTVENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-SKEDENFNLKHTCPGLLSMANAGENTNGSQFFITLAPCPS
PhvCYP8 1 -----MAKKKNSLVFMDVSTIGDGPVERIVIQLEASVPTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-DKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
PhvCYP9 1 -----DSEGVTNKVFEDVSTIGHGKLAGRIVIGLGNTPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-EKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
PhvCYP10 1 -----ELKEVTNKVFEDVSTIGKAAAGRIEIGLNGKAVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-SKADENFNLKHTCPGLLSMANAGENTNGSQFFITVTTSW
PhvCYP11 1 -----MSKNPKVFEDMTIGKMKAGRIVMELADVTPTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG---AFHRNIRNFMIQGGDFAGNCTGGESIYG-KPFKDELSKLVHSCRGVLSMANAGENTNGSQFFILYKSANH
PhvCYP12 1 MGKKQHSKDRMFIVKVEKNPKKKGYVQH-H-TTHGDNIELHCDIAPRACENFILTGER---AFHRNIRNFMIQGGDFAGNCTGGESIYG-KPFKDELSKLVHSCRGVLSMANAGENTNGSQFFILYKSANH
PhvCYP13 1 -----ALISAISAEPIGISTQTKYGDIEFGFEPVAPRTVDFIKLVKLGG---IFHRNIRNFMIQGGDFAGNCTGGESIYG-TKGGTISLKKFNDIRESLKNARGLLSMANAGENTNGSQFFITVTTSW
PhvCYP14 1 -----MGRGRCFEDVSTIGGELEGRIVVELHDVVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-HKEDENFELKHERKGLLSMANAGENTNGSQFFITTRTSH
PhvCYP15 1 -----MGRGRCFEDVSTIGGELEGRIVVELHDVVPKTAENFRALCTGKGCFFHRVIPSFMCGGDFAGNCTGGESIYG-HKEDENFELKHERKGLLSMANAGENTNGSQFFITTRTSH

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Fig.4: Multiple sequence alignment of cyclophilins from *Glycine max* and *Phaseolus vulgaris* showing conserved regions.

DISCUSSION

This study examined the orthologous relationships between cyclophilins from *Glycine max* (soybean) and *Arabidopsis thaliana*, highlighting their evolutionary conservation and functional roles in stress responses. The findings revealed several key orthologous pairs between GmCYPs and *Arabidopsis* cyclophilins, particularly those involved in light, heat, drought, and pathogen stress responses. These conserved relationships suggest that cyclophilins are fundamental in mediating stress tolerance across plant species, reinforcing their crucial role in plant survival under environmental stress.

Key genes such as *GmCYP28*, *GmCYP14*, and *GmCYP12* in soybean are orthologous to *Arabidopsis* ATCYP20-3, known for its role in light-induced oxidative stress responses (Dominguez-Solis et al., 2008). This suggests that GmCYPs likely play similar roles in protecting soybean plants from oxidative damage under high-light conditions. Similarly, the orthology between *GmCYP25*, *GmCYP26*, with *Arabidopsis* ATCYP20-2 further emphasizes the conserved function of cyclophilins in light stress responses, particularly in regulating the plant's redox balance (Romano et al., 2004). Additionally, the identification of *GmCYP37* as an ortholog of *Arabidopsis* ATCYP57, a cyclophilin involved in pathogen defense against *Pseudomonas syringae* (Pogorelko et al., 2014), highlights the multifunctional role of cyclophilins in both abiotic and biotic stress responses. This suggests that *GmCYP37* may

contribute to pathogen defense in soybean, reflecting the broader role of cyclophilins in plant immunity.

In relation to drought stress, *GmCYP13*, *GmCYP15*, and *GmCYP8*, which are orthologous to *Arabidopsis* ATCYP18-2, ATCYP19-1, and ATCYP21-2 (Park et al., 2013; Lee et al., 2015; Liu et al., 2021), are likely to be involved in enhancing drought tolerance in soybean. The functional conservation of these cyclophilins across species underscores their potential for improving drought resistance in crops, which is essential for agricultural productivity in water-scarce environments.

Furthermore, *GmCYP42*'s orthology with *Arabidopsis* ATCYP18-1, known for its role in heat stress tolerance (Sakuma et al., 2006), suggests that *GmCYP42* may be associated with heat stress tolerance in soybean, a particularly relevant trait as extreme temperature events are becoming more frequent due to climate change. Identifying and characterizing cyclophilins involved in heat stress tolerance offers new opportunities for improving crop resilience to high temperatures.

Previous researchers suggested evolutionary divergence between *Glycine max* and *Phaseolus vulgaris*, both species underwent whole genome duplication (WGD) events, with an additional WGD event occurring in *Glycine max* approximately 10-13 million years ago, which was absent in *P. vulgaris* (Schmutz et al., 2010; Schmutz et al., 2014). This additional WGD in *Glycine*

max likely contributed to the expansion of its gene pool, enhancing its adaptability to various environmental conditions, including improved pathogen resistance and climate adaptability.

In summary, this study provides compelling evidence of the evolutionary conservation of cyclophilins and their functional roles in stress responses across *G. max* and *A. thaliana*. The identification of orthologous relationships between GmCYPs and *Arabidopsis* cyclophilins enhances our understanding of cyclophilin function and suggests that these proteins are crucial for improving stress tolerance in crops. Future research on the functional characterization of these cyclophilins in soybean and other leguminous crops could lead to the development of genetically modified varieties with enhanced resistance to environmental stresses, contributing to global food security in the face of climate change.

CONCLUSION

This study provided valuable insights into the orthologous relationships between *Glycine max* (soybean) cyclophilins and those of *Arabidopsis thaliana*, highlighting the evolutionary conservation of cyclophilin functions across species. By analyzing 28 GmCYPs, we identified several key orthologous relationships with *Arabidopsis* cyclophilins, many of which are involved in stress responses, such as light, heat, drought, and pathogen stress. These findings suggested that GmCYPs share functional similarities with their

Arabidopsis counterparts, potentially playing critical roles in enhancing stress resilience in soybean.

The study also underscores the evolutionary divergence between *Glycine max* and *Phaseolus vulgaris*, two important leguminous crops, following their split from a common ancestor approximately 19.2 million years ago. The whole genome duplication events that both species underwent, particularly the additional WGD event in *Glycine max*, could have contributed to the diversification of their genomes, enabling these species to adapt to a variety of environmental conditions. The additional WGD in *Glycine max* may have played a significant role in expanding the gene pool, facilitating the adaptation of soybean to diverse environmental stresses, and enhancing its agricultural productivity.

Overall, the orthologous relationships identified in this study provided a deeper understanding of the functional conservation and divergence of cyclophilins across leguminous crops and model species. The results emphasized the potential of GmCYPs in soybean to serve as targets for improving stress tolerance, thus enhancing the resilience and productivity of soybean crops in changing environmental conditions.

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Ansari M M and Gupta G K. 1999. Epidemiological studies of foliar diseases of soybean in Malwa plateau of India. *Proceedings, World Soybean Research Conference VI, Aug 4-7, 1999, Chicago, Illinois, USA*, 611p. **(Symposium/ Conf./Workshop)**

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Opening Balance	5,903,455	Computer & Printer	5
		Less :- Depreciation	2
Add : Surplus of the year	75,550		3
	5,979,005	Furniture & Fixture	7,157
		Less :- Depreciation	716
			6,442
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		Fixed Deposit	1,098,800
		Accrued Interest	116,523
		Mutual Fund	4,333,860
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